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First Edition . . 1938
Second Edition . 1940
Reprinted . 1941
Third Edition . 1943
Reprinted . 1944
Reprinted . 1946
Fourth Edition . 1947

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PREFACE TO THE FOURTH EDITION

The issue of a new edition is necessary to keep pace with the recent advances in our knowledge of Biochemistry. A new chapter on the Use of Isotopes in Biochemical Investigations, which could not be included in the previous edition owing to paper restrictions, has now been added. Much other new material has been incorporated and the sections on protein structure, co-enzymes, flavoproteins, bile pigments and nutrition in wartime have been largely rewritten.

I am very greatly indebted to my colleague Dr. H. G. Bray for many helpful suggestions and for reading the revised manuscript and the proofs, and I wish to thank Messrs. J. & A. Churchill for providing facilities for the revision.

W. V. THORPE.

PREFACE TO THE FIRST EDITION

The aim of this book is to present an account of the biochemical processes known to occur in the healthy human body. Knowledge in this field has so increased during recent years that many facts of interest to the medical and dental student cannot now be included within the compass of the recommended text-books of Physiology This book is intended to be complementary to these text-books, and subjects like respiration, which are fully described there, have in consequence not been included in this volume.

In view of their importance in medical diagnosis, prominence has been given to blood, urine and fæces. Special attention has been paid to the principles of nutrition and the composition of foodstuffs, since these aspects of Biochemistry are not usually accorded the space which their importance merits. I hope that the presentation of digestion, absorption and metabolism from the biochemist's point of view may help the student to see the biochemical and physiological aspects of these subjects in true perspective. Many students are prone to explain away difficulties by the too glib use of the term "physico-chemical." This is my reason for an extended discussion of the physico-chemical aspects of Biochemistry and of absorption in particular.

I am well aware of the small amount of time which most medical students are able to devote to the study of Biochemistry, and I have accordingly taken pains to be as concise as is consistent with accuracy. I have tried to provide what the medical or dental student may want to know and to omit that which he is never likely to require. The wide variation in the emphasis laid upon the study of Biochemistry in different medical schools means that some readers may find more in this book than they really need. The subject-matter has been arranged in short sections, some of which can be omitted at the direction of the teacher. For those whose interest carries them beyond the confines of this book, a bibliography has been provided on pp. 470–473, to which references have been made from the appropriate places in the text. The greater part of the text has been based upon material in these books and articles, and I wish to acknowledge my indebtedness to the authors.

One pleasant aspect of writing this book has been the generous help I have received from my friends. Professor A. Wormall, who was responsible for persuading me to undertake the work, Dr. Esther M. Killick, and my colleague, Dr. R. T. Williams, read the typescript and suggested many corrections and improvements. My wife corrected the manuscript and proofs at each stage and assisted me in compiling the index. To these four I find it very difficult to express my gratitude, for they have contributed much to any accuracy and clarity this book may have. Others whose help it is a pleasure to acknowledge are Dr. K. H. Coward, for reading the sections on vitamins and providing some values for the vitamin tables; Miss I. O'Brien, for reading a considerable part of the manuscript; and Professor H. P. Gilding, for advice on a number of physiological points.

On the technical side I have been equally fortunate. I am much indebted to my laboratory steward, Mr. L. Evans, for the preparation of photographs and drawings for most of the illustrations and over 100 lay-outs of formulæ. The efficient typing of Miss M. Butler lightened the task of the preparation of the manuscript for the printers. To Messrs. Churchill, and in particular Mr. J. Rivers, who have been most courteous and helpful throughout, I wish to offer my thanks.

For permission to reproduce illustrations or use data from their published works, my best thanks are due to Dr. M. A. Boas Fixsen, Dr. K. H. Coward, Dr. G. A. Harrison, Professor V. H. Mottram, Sir John B. Orr, Professor F. Verzár and Dr. D. M. Wrinch. Acknowledgement has been made in the appropriate places in the text. Messrs. Longmans, Green & Co. have kindly loaned the blocks for Figs. 18 and 21, and Messrs. Macmillan granted permission for reproduction of Figs. 30 and 31.

W. V. THORPE.

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BIOCHEMISTRY FOR MEDICAL STUDENTS

PART I

CHAPTER I

INTRODUCTION

Biochemistry is the study of chemical processes occurring in *living* tissues and not, as some chemists believe, the Chemistry of Natural Products, that is, Organic Chemistry in its original sense. The chemistry of dead meat and withered flowers only concerns Biochemistry in that it gives approach to the nature of the *living* animal or plant. The details of the laboratory synthesis of a natural compound do not concern Biochemistry unless there is reason to suppose that the laboratory process imitates the natural one. The professional biochemist is interested in such a synthesis because he has an æsthetic appreciation of molecular architecture which makes him an admirer of the art of the organic chemist ; but a medical or dental student should not be expected to appreciate a masterpiece of chemical synthesis any more than a chemist to appreciate details of surgical technique. It is for this reason that this book, which has been written especially for Medical and Dental students, contains no details of laboratory syntheses.

Chemical formulæ, however, have been used freely because they provide the only concise method of describing compounds, and a knowledge of the nature of a compound is of assistance in understanding its properties and, therefore, the changes it undergoes in the body. A chemical formula is something more than a shorthand symbol. It not only gives a rough picture of the arrangement of the atoms in a compound but also an indication of how the compound will behave (*i.e.*, whether it is an acid, alcohol, base, etc.), what substances it could be changed into, and from what compounds it could be formed. Chemical formulæ are included in this book solely in order to explain chemical changes more clearly than is possible in words. The first aim of the student should be to understand a formula by looking for characteristic groups and arrangements, making no attempt to memorise it as a pattern ; later, if examination conditions demand it, formulæ will be found easy to memorise.

The Biochemistry required by Medical and Dental students forms the link between Chemistry and Human Physiology. The connection between Biochemistry and Human Physiology is such an intimate one that the two subjects must be studied *side by side* if full benefit is to be got from either. The subjects are usually taught separately for technical reasons ; they are so vast that the average biochemist or physiologist has not the capacity for a comprehensive knowledge of both.

Just as Human Physiology is essentially the study of the functioning of the normal healthy man, and only deals with disease in so far as it helps to clarify the normal function, so here abnormal Biochemistry (Pathological or Clinical Chemistry) will only be discussed when it helps to reveal the normal. For the clinical application of Biochemistry the reader is recommended to Harrison's " Chemical Methods in Clinical Medicine " (Ref. 3) or Peters and van Slyke's "Quantitative Clinical Chemistry" (Ref. 8).

The human body contains a vast array of compounds, many of which are present only in minute amount ; the nature of many of these is still unknown. We do not even know how many different substances there are.

Elementary Composition of the Body

Elementary analysis of the human body reveals the presence of about 64% oxygen, 18·5% carbon and 9·9% hydrogen. The bulk of the oxygen and hydrogen is present in the form of water which forms 65% of the body. Of the remaining 35% most is organic matter, as the following table shows :—

AVERAGE ELEMENTARY COMPOSITION OF THE SOLIDS OF THE HUMAN BODY (expressed as per cent. of total body weight.)

Carbon	18·5	Chlorine	0·16
Oxygen	6·5	Sulphur	0·14
Hydrogen	2·7	Potassium	0·10
Nitrogen	2·6	Sodium	0·10
Calcium	2·5	Magnesium	0·07
Phosphorus	1·1	Iron	0·0

Copper, manganese and iodine are present in very small amounts. All these elements are essential for life. Al, As, Br, F, Si and Zn are always present, although there is no evidence of any special function. Traces of other elements are also frequently found.

Of these elements only oxygen, nitrogen and hydrogen exist in elementary form (a small amount of hydrogen is formed by putrefaction in the large intestine). The compounds of these and the other elements can be classified into groups of substances with similar properties. The fat-like substances soluble in solvents like ether and alcohol are called *lipides* ; the bulk of the residue is composed of nitrogenous compounds called *proteins* * ; the remainder consists of *carbohydrates*, *other organic substances* and *inorganic substances*. The last are frequently estimated by burning away the organic substances, leaving the *ash* (the metals as oxides, chlorides, phosphates or sulphates).

Proteins, although the most abundant, are not the only nitrogenous constituents of the body. This will be clear if we classify the substances in another way, thus :—

Inorganic Substances	Organic Substances	
	(*a*) Nitrogenous	(*b*) Non-nitrogenous
Water . . . . . Salts . . . . . Other substances (ammonia, hydrochloric acid, free oxygen, etc.).	Proteins. Certain lipides. Other substances (urea, creatine, purines, etc.).	The other lipides Carbohydrates. Other substances (glycerol, lactic acid, etc.).

Plant tissues qualitatively resemble animal tissues in containing compounds which would fall into the same groups, although the individual substances are not identical ; but quantitatively the results of analyses are very different, as seen in the table on p. 4.

---

* The amount of non-protein nitrogenous compounds in this residue is so small that proteins can be roughly estimated by multiplying the total nitrogen by a factor.

COMPARISON OF ANIMAL AND PLANT TISSUES

Percentage of :—	Water	Fat	Protein	Carbohydrate	Ash
Ox liver . . .	71·2	4·5	20·7	1·5	1·6
Ox muscle . .	75·9	0·9	18·4	1·5	1·3
Cabbage leaf . .	89·2	0·4	1·8	6·9	1·3
Potato . . .	78·3	0·1	2·2	18·0	1·0

Notice the difference in protein and carbohydrate. In animals proteins form the chief supporting tissues, whereas in plants we find mainly carbohydrates. Plants contain relatively little fat. And yet man can feed entirely upon plant food, do work and maintain his own tissues therefrom. The study of Biochemistry is an attempt to find out the chemical reactions involved in these processes.

Plan of this Book

All living processes are carried out in aqueous solution, so that as a preliminary the *physico-chemical principles* involved must be briefly reviewed (Chaps. II–IV). Next we must study the commoner *substances found in our tissues and food* (Chaps. V–IX) ; other substances of physiological importance are described in Chapters XII, XIII, XXI, XXVII, XXVIII. Living tissues are provided with *special catalysts* which facilitate and control various chemical reactions (Chaps. X, XI). The *distribution* of the different substances in our tissues is given in Chapters XII and XIII.

In Part II details of the better-known metabolic processes are discussed, showing how food is *digested* and *absorbed* into the body and changes which substances undergo in the tissues—*intermediary metabolism* (Chaps. XIV–XXV). Many of these processes are still very obscure, for the elucidation of metabolic processes *in vivo* is beset with experimental difficulties.

Chap. XXVI describes how the body uses atmospheric oxygen and excretes carbon dioxide.

Several substances, although present only in minute amounts, have a powerful *controlling influence* on metabolic processes (Hormones and Vitamins, Chaps. XXVII, XXVIII).

In Part III we study the body as a *whole*, its *energy needs*

(Chap. XXIX), the form and conditions under which food should be taken (Chap. XXX) and the composition of food (Chap. XXXI). Finally, we examine the *waste products* eliminated by the body (Chap. XXXII). Some miscellaneous information is recorded in the appendix.

In a subject like Biochemistry it is impossible to give a complete account of a particular process without either breaking the thread by pausing to describe the compounds concerned or repeating some details which have already been described. These compounds have as far as possible been relegated to the earlier chapters and repetitions eliminated by cross references. Care has been taken to provide a comprehensive index, so that all facts relating to a particular substance or process can be quickly collected. The value of frequent use of the index for revision cannot be too strongly emphasised.

One further point may be mentioned here. It has been the author's experience that many students are frequently puzzled by apparent discrepancies of fact because they are in the habit of thinking qualitatively rather than quantitatively. Many difficulties are solved by considering a problem quantitatively, not only in the sense of actual weights reacting, but also the *time* in which they are able to react. In other words it will be found helpful to acquire the mental habit of seeing details against a background of the whole. All chemical mechanisms which are elucidated must fit quantitatively as well as qualitatively against the background of the *normal intact animal* before they can be finally accepted as correct. In the following pages the reader will perceive how far we are from this ideal in most known biochemical processes.

CHAPTER II

ACIDITY AND ALKALINITY (5, 11, 12, 14)

WITH the exception of gastric juice, the reaction of the tissues and tissue fluids of the body is nearly neutral. We know that the tissues are very sensitive to the reaction of the fluids bathing them, and slight changes in reaction may have a profound physiological effect. Consider, for example, an isolated frog's heart which beats continuously if perfused with a solution of the right composition and reaction; if, however, the reaction of the solution is made ever so slightly more acid or more alkaline the heart stops. Every biochemical reaction in living tissue has a definite acidity or alkalinity optimum.

It is therefore necessary that we should have some means of defining small changes in acidity or alkalinity, for it is not sufficient to distinguish between acid or alkaline, as the following example will show. Twelve different solutions could easily be prepared in which the acidity or alkalinity is ideal for each of twelve biochemical reactions. If we tested them for acidity or alkalinity with indicators we might find the solutions behaving somewhat like this :—

Indicator	Number of Solutions reacting Acid	Number of Solutions reacting Alkaline
Methyl orange . .	3	9
Methyl red . . .	6	6
Phenol red . . .	7	5
Phenolphthalein . .	12	0

Obviously we must attempt to define acidity accurately and numerically. The *normality* system will not avail here, for it merely tells us the quantity of acid which will combine with a given amount of base. It does not distinguish between N/10 HCl and N/10 acetic acid, a distinction which is only too obvious to the taste buds of the tongue. (Vinegar contains about seven times as much acetic acid as an N/10 solution and yet tastes less acid than N/10 HCl.) What we want to define is not the quantity of acid present, but the *intensity* of the acidity of a solution regardless of the amount of acid present or its chemical nature.

Acidity is caused by an excess of hydrogen ions, ($H^{\cdot}$), over hydroxyl ions, (OH'),* and alkalinity by an excess of OH' over $H^{\cdot}$. The intensity of acidity depends upon the amount of hydrogen ions in excess, and the intensity of alkalinity is proportional to the excess hydroxyl ions. If the amounts of hydrogen and hydroxyl ions present are the same the solution is neutral. Pure water is a typical neutral solution, and it contains $H^{\cdot}$ and OH' in equal proportions. It is not free from $H^{\cdot}$ and OH'. These ions are formed by ionisation, thus :—

$$H_2O \rightleftharpoons H^{\cdot} + OH'$$

However much ionisation there is the concentration of $H^{\cdot}$ is always equal to the concentration of OH', for if $H^{\cdot}$ is split off OH' remains.

We can increase the concentration of $H^{\cdot}$ without increasing OH' by adding an acid which ionises to form $H^{\cdot}$, *e.g.*,

$$HCl \rightleftharpoons H^{\cdot} + Cl'.$$

Similarly we can increase the concentration of OH' without increasing $H^{\cdot}$ by adding an alkali, *e.g.*, $NaOH \rightleftharpoons Na^{\cdot} + OH'$.

It must be remembered, however, that even in the most acid solutions there are always some OH' and conversely in alkaline solutions there are always some $H^{\cdot}$. Aqueous solutions always contain both $H^{\cdot}$ and OH' because water always ionises to some extent.

A solution is acid because there are more $H^{\cdot}$ *than* OH'.

A solution is alkaline because there are more OH' *than* $H^{\cdot}$.

Now the ionisation of water is a reversible reaction obeying the Law of Mass Action. That means that the product of the concentrations of $H^{\cdot}$ and OH' bears a constant ratio to the concentration of water in any system in equilibrium containing these three components, or, as it is usually expressed :—

$$[H^{\cdot}] \times [OH'] = K[H_2O] \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

---

* $H^{\cdot}$ and OH' can also be written H^+ and OH^-.

where K is a constant and square brackets signify the concentration.

If we add $H^{\cdot}$ to this system, we shall increase the left-hand side of the equation. Therefore, in order to re-establish equilibrium, OH' must decrease by combining with $H^{\cdot}$ to form water until the equation is balanced. In other words, if $H^{\cdot}$ are added to a solution, the ionisation of the water will be depressed and there will be fewer OH' than in pure water. Similarly, addition of OH' will depress the ionisation of water and diminish the concentration of $H^{\cdot}$.

Actually water is only ionised to such a small extent that even if all the $H^{\cdot}$ and OH' recombined to form water the increase in the concentration of water molecules would be negligible. Therefore, when considering the effect of adding $H^{\cdot}$ or OH' to a system, we can neglect the fact that the addition will cause a minute increase in the concentration of H_2O. As we shall see later 1 g. of $H^{\cdot}$ and 17 g. of OH' are contained in 10,000,000 litres of water; complete depression of ionisation would therefore only increase the amount of water from 10,000,000,000 to 10,000,000,018 g. We can therefore take the concentration of water as constant, and rewrite equation (1):

$$[H^{\cdot}] \times [OH'] = K \times c = K_w \quad . \quad . \quad . \quad . \quad . \quad (2)$$

The constant K_w is called the *ionic product* of water. It must be clear that equation (2) applies to any system containing water, $H^{\cdot}$ and OH', *i.e.*, to pure water and *all* aqueous solutions. In an acid solution $[H^{\cdot}]$ is large. Therefore $[OH']$ is small, but never zero, for $[H^{\cdot}] \times 0 = 0$, which does not $= K_w$. Similarly in an alkaline solution $[H^{\cdot}]$ is never zero. To give a numerical example, in *any* aqueous solution if $[H^{\cdot}]$ is doubled, $[OH']$ will be halved to establish equilibrium.

$$2[H^{\cdot}] \times \tfrac{1}{2}[OH'] = K_w = [H^{\cdot}] \times [OH']$$

In pure water, or a perfectly neutral solution, $[H^{\cdot}] = [OH']$, so that equation (2) may be written $[H^{\cdot}]^2 = K_w$.

We can find $[H^{\cdot}]$ of pure water by measuring its electrical conductivity. In this way it is found that the concentration of $H^{\cdot}$, $[H^{\cdot}]$, or, as more usually expressed biologically, cH, is one ten-millionth Normal at 22° C. That means 1 litre of pure water contains

$$\frac{1}{10000000} = \frac{1}{10^7} = 10^{-7} \text{ g. of } H^{\cdot}.$$

Hence not only cH but cOH $= 10^{-7}$ N and $K_w = (10^{-7})^2 = 10^{-14}$.

Now a solution of N/1,000 HCl, in which the HCl is 100% ionised, would have a hydrogen ion concentration of N/1,000, *i.e.*, cH 10^{-3}, neglecting the very small amount of $H^{\cdot}$ due to ionisation of water. Since cH × cOH = 10^{-14}, N/1,000 HCl will have a cOH 10^{-11}. Similarly N/10,000 NaOH will have cOH 10^{-4} and cH 10^{-10}. It follows therefore that we can express the acidity or alkalinity of a solution by giving its cH (or cOH) alone.

If cH is greater than 10^{-7} (*e.g.*, 10^{-5}) *the solution is acid.*
If cH is less than 10^{-7} (*e.g.*, 10^{-12}) *the solution is alkaline.*

In the examples quoted above, HCl and NaOH are practically 100% ionised. Solutions of these substances are, in fact, almost completely ionised in solutions more dilute than N/10, so that below this concentration we can state the cH if we know the normality of the acid or alkali. At concentrations greater than this ionisation is not complete, and we cannot state cH until we know the degree of ionisation. With weak acids, such as acetic, 100% dissociation is only attained in solutions considerably weaker than N/10. That is why N/10 acetic acid is so much less acid than N/10 HCl, for N/10 acetic acid is only 1·35% ionised and has, therefore, a cH of $1{\cdot}35 \times 10^{-3}$.

The reactions of all biological fluids lie between about cH 10^{-1} and 10^{-10}, and since such expressions are somewhat cumbersome for continual reference it is customary to use Sørensen's pH notation and write only the negative power after the symbol pH, *e.g.*, cH 10^{-7} = pH 7.

The pH of a solution is the negative value of the power to which 10 *must be raised to give the actual cH in grams per litre ; or expressed mathematically* $pH = \log \frac{1}{cH} = -\log cH$.

A solution of pH 3 contains 10^{-3} g. $H^{\cdot}$ per litre.
A solution of pH 5 contains 10^{-5} g. $H^{\cdot}$ per litre.
A solution of pH 8 contains 10^{-8} g. $H^{\cdot}$ per litre.

Note that a solution of pH 3 has ten times the hydrogen ion concentration of one of pH 4 and a hundred times that of a solution of pH 5. As cH increases pH *decreases* in such a way that every decrease of one unit in pH means cH has increased 10 times. A neutral solution has pH 7. A solution with pH less than 7 is acid, and greater than 7 alkaline. It must be remembered that the pH scale is logarithmic, not numerical, *e.g.*, pH 6·5 does not represent a cH half-way between pH 6 and pH 7. Actually pH 6·5 = cH $3{\cdot}2 \times 10^{-7}$; pH 6 = cH 10×10^{-7} = cH 10^{-6}.

pH may be readily calculated from a given cH, and cH from a given pH, as in the following examples :—

Calculation of the pH of a solution of cH $2{\cdot}3 \times 10^{-4}$.

$$\text{pH} = \log \frac{1}{\text{cH}} \qquad \therefore\ \text{pH} = \log \frac{1}{2{\cdot}3 \times 10^{-4}}$$

$$= \log 1 - \log (2{\cdot}3 \times 10^{-4}).$$

$$\text{Since } \log 1 = 0, \qquad \therefore\ \text{pH} = -\log 2{\cdot}3 - \log 10^{-4}$$

$$= -0{\cdot}3617 + 4$$

$$= 3{\cdot}64 \text{ to the nearest two places.}$$

Calculation of the cH of a solution of pH 8·3.

$$\text{pH} = 8{\cdot}3 = 9 - 0{\cdot}7$$

$$= 9 - \log 5{\cdot}01$$

$$= \log \frac{1}{5{\cdot}01 \times 10^{-9}}$$

$$\therefore\ \text{cH} = 5{\cdot}01 \times 10^{-9}.$$

The table gives the pH of some typical solutions.

Solution	pH	Solution	pH
N/10 hydrochloric acid	1·04	Pure water	7·0
Gastric juice	1·4	Milk	7·1
N/100 hydrochloric acid	2·0	Tears	7·2
N/10 acetic acid	2·87	Blood	7·4
Infants' gastric juice	5·0	Intestinal juice	7·8
Urine (average)	6·0	Pancreatic juice	8·0
Saliva	6·8	N/100 NaOH	12·0

It should be noted that the pH of a solution may vary with temperature, since ionisation is increased on raising the temperature. Pure water at 22° C. has pH 7; but at 38° C. it has pH 6·74. If a solution is completely ionised, *e.g.*, N/10 HCl the pH will not vary with the temperature.

INDICATORS

The concentration of hydrogen ions in a solution can be determined by means of certain dyes called indicators. These dyes are weak acids (or less commonly weak bases) which have the property of dissociating * in solution to give, in effect, ions of a different colour from the undissociated molecule.[*] The pH at which dissociation will occur depends upon the strength of the acid ; the stronger the acid the lower the pH at which it dissociates. We can represent the free acid of methyl orange, which is red, as MH. On dissolving in water dissociation will occur thus :

---

* Ionisation is a special type of dissociation, electrolytic or ionic dissociation. In physico-chemical usage the adjectives are omitted where the sense is obvious and the terms ionisation and dissociation are synonymous. The expression "dissociation into ions" is more euphonious than "ionisation into ions."

$$MH \rightleftharpoons H^{\cdot} + M'$$

M' being a yellow ion. The colour of the solution will depend upon the extent of ionisation, so that in very dilute solution it will be yellow. In stronger solutions with incomplete dissociation it may be orange or with no dissociation red. Now if we add acid (*i.e.*, $H^{\cdot}$) we shall depress the ionisation and reform MH and the colour will be red. If we add alkali (*i.e.*, OH') the OH' will combine with $H^{\cdot}$ to form water, so that MH will ionise to maximum extent and the colour will be yellow.

Indicator	pH Range	Colour-Change
†Thymol blue (first)	1·2–2·8	Red → Yellow
Töpfer's reagent	2·9–4·0	Red → Yellow
Methyl orange	3·1–4·4	Red → Yellow
†Bromphenol blue	3·0–4·6	Yellow → Blue
Congo red	3·0–5·0	Blue → Red
†Bromcresol green	4·0–5·6	Yellow → Blue-green
†Methyl red	4·3–6·3	Red → Yellow
†Bromcresol purple	5·2–6·8	Yellow → Purple
†Bromthymol blue	6·0–7·6	Yellow → Blue
†Phenol red	6·8–8·4	Yellow → Red
†Cresol red	7·2–8·8	Yellow → Red
†Thymol blue (second)	8·0–9·6	Yellow → Blue
†Phenolphthalein	8·3–10·0	Colourless → Red

† Suitable for pH determination.

The use of indicators is not restricted to determining the titratable acidity (or alkalinity), that is, the total amount of $H^{\cdot}$ (or OH') the solution can yield on complete dissociation. If we have available a range of indicators which are acids of gradually diminishing strength, *i.e.*, a series of dyes which dissociate over the whole pH range (the dissociation constants of a vast number of dyes have been determined to this end), we can obtain a rough indication of the pH of a solution by adding just sufficient dye to colour the solution. When we find an indicator gives its intermediate colour (*i.e.*, it is partially dissociated) we can say that the pH of the solution is about the middle of the range (highest pH at which it is unionised to pH of complete dissociation) of the indicator. (For accurate determination, see p. 16.) It is important that the dye should have good colouring power, for we must not add a large amount of dye, since it is an acid which might change the pH if more than traces were present. The table gives a list of indicators especially suitable for pH determination (marked †) and some other common indicators useful for the biological pH range.

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[*] To be accurate, the formation of a coloured ion directly from a colourless or differently coloured acid is most unlikely. Before or after dissociating, the indicator acid must be assumed to undergo a tautomeric change to an acid of different colour. For practical purposes the ultimate effect is as if the acid dissociated to give an ion of a different colour, and as such we will consider it.

THE TITRATION OF ACID AND ALKALI

The titration of an acid with an alkali is only a simple process when stable (or insoluble) salts are formed, as with strong acids and strong bases. Only then does the mixture of equal volumes of, say, N/10 acid and alkali result in a neutral solution. In this case either methyl orange or phenolphthalein (or equivalent indicators) could be used, because the change of pH approaching neutrality is so rapid that one drop of N/10 acid or alkali works the change, although theoretically an indicator with a range including pH 7 should be used. If, however, equal volumes of N/10 acetic acid and N/10 NaOH are mixed, the resulting solution is not neutral but alkaline. This is because sodium acetate undergoes hydrolysis in solution ; this can be represented ionically :

$$Na^{\cdot} + Ac' + H^{\cdot} + OH' \rightleftharpoons Na^{\cdot} + OH' + HAc.$$

The solution is alkaline because acetic acid is not completely ionised, so that OH′ ions are left in excess. With HCl, which is completely dissociated, no $H^{\cdot}$ are removed by Cl′ and so there is no excess of OH′.

Conversely with a weak base and strong acid, *e.g.*, $NH_4OH + HCl$, we have

$$NH_4^{\cdot} + Cl' + H^{\cdot} + OH' \rightleftharpoons NH_4OH + Cl' + H^{\cdot}.$$

Therefore in titrating acetic acid with NaOH we must choose an indicator which only changes with a concentration of OH′ greater than would be present in a solution of sodium acetate, *i.e.*, an indicator which dissociates at a pH more alkaline than that of sodium acetate, *e.g.*, phenolphthalein. Similarly an indicator dissociating at low pH must be chosen for the titration of ammonia with HCl, *e.g.*, methyl orange.

BUFFER SOLUTIONS

Many reactions in the tissues involve the liberation of acid (or base) and yet one of the most characteristic features of living tissues is the extraordinarily narrow limits within which the pH varies. The pH of human blood is about 7·4, and values of 7·0 and 7·6 are extremely dangerous, if not fatal. In health it lies between 7·3 and 7·5, in spite of the fact that CO_2 (*i.e.*, carbonic acid) is always being added. The difference between arterial and venous blood is rarely more than 0·04 pH. In severe muscular exercise it is only when the blood lactic acid content rises over 100 mg. per 100 c.c. that an appreciable decrease in blood pH is observed. The power of the blood to absorb acid (or base) without appreciable change in pH is due to its content of substances which act as " buffers." To explain this term it will be simplest to study the effect of adding acid to some simple salt solutions.

We could have three solutions of pH 7, as follows :—

(*a*) Pure water.

(*b*) A solution of NaCl.

(*c*) A solution of KH_2PO_4 mixed with an appropriate amount of NaOH.

If we take 100 c.c. of each of these solutions and add an indicator such as bromcresol purple (pH 5·2–6·8) and 1 c.c. N/100 HCl, (*a*) and (*b*) will turn yellow, indicating a pH less than 5·2, whereas (*c*) will not change colour until a considerable quantity of acid has been added.

A solution such as (*c*) which tends to maintain its pH in spite of addition of moderate amounts of acid or alkali, is called a "*buffer*" solution. In contrast with solutions of acid or base alone, buffer solutions do not change their pH on dilution. Buffers act as shock absorbers against sudden changes of pH. In general, buffer solutions are composed of mixtures either of a weak acid and its salt with a strong base, or of a weak base and its salt with a strong acid. We can represent the composition of a buffer solution made from a salt, BA, of an acid, HA :—

$$\begin{cases} HA \rightleftharpoons H^{\cdot} + A' & \ldots\ldots\ldots (a) \\ BA \rightleftharpoons B^{\cdot} + A' & \ldots\ldots\ldots (b) \end{cases}$$

On addition of alkali we shall have

$$Na^{\cdot} + OH' + H^{\cdot} + A' \rightarrow Na^{\cdot} + A' + H_2O \quad . \quad . \quad . \quad (c)$$

from (*a*), and on addition of acid

$$B^{\cdot} + A' + H^{\cdot} + Cl' \rightarrow B^{\cdot} + Cl' + HA \quad . \quad . \quad . \quad . \quad (d)$$

from (*b*), for HA is a weak acid. In neither case will there be anything like the pH change there would be if the alkali or acid were added to water. In practice the buffer mixture is always present in relatively large excess so that the pH change is negligible.

By proper choice of the weak acid or base a buffer solution may be prepared for any desired pH. Applying the Law of Mass Action to the acid we have

$$[H^{\cdot}] \times [A'] = K[HA] \qquad \therefore [H^{\cdot}] = K\frac{[HA]}{[A']}$$

where K is the ionisation constant of the acid.

Since HA is a weak acid and hardly ionised, we can say that [HA] in the equation = the *total* acid present, and since A' in the buffer solution is almost entirely from the salt BA and not the acid, we can say that $[A']$ = *total* salt present. We have therefore

$$[H^{\cdot}] = K\frac{[\text{Acid}]}{[\text{Salt}]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (e)$$

If we have equal parts acid and salt, $[H^{\cdot}]$ will be equal to the dissociation constant K of the acid. In other cases it will be a multiple or fraction of K. We have assumed that the salt is completely dissociated. If the solution is not sufficiently dilute for almost complete dissociation the equation (*e*) becomes

$$[H^{\cdot}] = K\frac{[\text{Acid}]}{\alpha[\text{Salt}]} = K_1\frac{[\text{Acid}]}{[\text{Salt}]} \quad . \quad . \quad . \quad . \quad . \quad (f)$$

where a is the degree of dissociation of the salt (and practically constant).

Now let us consider some typical buffer systems. We will write these as chemical equations. The ionisation equations can easily be written out by substituting in equations (*a*), (*b*), (*c*) and (*d*).

(1) *Sodium Acetate + Acetic acid.*

Adding acid, $HAc + NaAc + HCl \rightarrow 2HAc + NaCl$.
Adding alkali, $HAc + NaAc + NaOH \rightarrow 2NaAc + H_2O$.

(2) *Sodium or Potassium Phosphates.*

Adding acid, $NaH_2PO_4 + Na_2HPO_4 + HCl \rightarrow 2NaH_2PO_4 + NaCl$.
Adding alkali, $NaH_2PO_4 + Na_2HPO_4 + NaOH \rightarrow 2Na_2HPO_4 + H_2O$.

(In this case NaH_2PO_4 can be considered as the acid, ionising to $Na^{\cdot}$, $H^{\cdot}$ and HPO_4'', and Na_2HPO_4 as the salt.)

(3) *Sodium Bicarbonate + Carbonic Acid.*

Adding acid, $H_2CO_3 + NaHCO_3 + HCl \rightarrow 2H_2CO_3 + NaCl$.
Adding alkali, $H_2CO_3 + NaHCO_3 + NaOH \rightarrow 2NaHCO_3 + H_2O$.

When it is remembered that the buffer is present in excess it will be realised that the addition of acid or alkali in these cases will cause little change in pH.

Most tissue and intracellular fluids are buffer solutions containing several buffer pairs (*i.e.*, weak acid + salt). This is of importance, for the catalysts which control the cellular reactions only work within a narrow range of pH, so that acid or base formed during a reaction must be effectively buffered if the reaction is to proceed to any extent. The constancy of the pH of blood has already been remarked upon. The blood has to transport acids and bases *e.g.*, acidic and basic amino-acids, fatty acids, phosphoric acid, sulphuric acid, formed by digestion of food and carbonic acid and lactic acid formed by oxidation in the tissues. The chief buffer pairs in the blood are :—

H_2CO_3	H. Hæmoglobin	H. Oxyhæmoglobin	BH_2PO_4	H. Protein.
$BHCO_3$,	B. Hæmoglobin,	B. Oxyhæmoglobin,	B_2HPO_4,	B. Protein.
6·1	7·3	7·16	6·8	

The figures below each pair indicate the pH of the buffer at maximum efficiency (*i.e.*, when $\frac{[\text{acid}]}{[\text{salt}]} = 1$) calculated from equation (*f*). Note that all are more acid than blood ; this means that the efficiency of the blood buffers against acids increases if the pH falls. In blood at pH 7·4 the ratio $H_2CO_3 : BHCO_3$ is 1 : 20 and $BH_2PO_4 : B_2HPO_4$ 1 : 3·55. Actually in blood the buffering of carbonic acid is complicated by the presence of the red cells, see Chap. XXVI.

DETERMINATION OF pH

(*a*) *Colorimetric Methods.*

(1) The simplest way of estimating the pH of an unknown is to find the approximate pH by spot tests with indicators. A suitable salt of a weak acid is then selected and titrated with alkali (or acid) until the pH matches that of the unknown, both solutions being coloured with the same concentration of a suitable indicator. Tables are available showing the pH of certain salt solutions when treated with varying volumes of acid or alkali, so that the pH of the unknown solution can be read off. Buffer mixtures commonly used are :—

Buffer Series.	pH Range.
Glycine and HCl	1·2– 3·6
Acid potassium phthalate and HCl	2·2– 3·8
Acid potassium phthalate and NaOH	4·0– 6·2
Acid potassium phosphate and NaOH	5·8– 8·0
Boric acid, HCl and NaOH	7·8–10
Glycine and NaOH	8·6–12·9

By suitable mixture of salts and acids it is possible to make buffer mixtures covering practically the whole pH range. A mixture of phosphoric acid, acetic acid, boric acid and NaOH will buffer from pH 2–12.

(2) A method more generally used consists in having made up in sealed glass tubes buffers (with suitable indicators) for every 0·2 pH. The unknown is treated with sufficient indicator to give the same concentration as in the standard tubes and matched against the standards. While this method has the advantage of speed and economy of solution over method (1) it suffers from the disadvantage that the standard tubes are difficult to keep for any length of time owing to the gradual solution of alkali from the glass and other reasons. The large stock of standard tubes required makes the method more suitable for continuous than occasional use.

Other colorimetric methods obviating the use of buffers are described in practical textbooks.

(*b*) *Electrometric Methods.*

(1) **Hydrogen Electrode.** If a strip of metal dips into water it tends to dissolve. Metal goes into solution as positive ions leaving negatively charged electrons on the metal. This charge attracts the positive metal ions so that no more metal dissolves. Equilibrium is established before a detectable amount of metal goes into solution. Nevertheless, the charges on the ions are sufficiently large to cause a measurable potential difference between the metal and the water. If the metal dips into a solution of one of its salts, the metal ions already in solution oppose the separation of ions from the metal. Equilibrium will be established when the electrolytic solution pressure P of the metal equals the osmotic pressure p of its ions in solution. The potential difference E between a metal and a solution of one of its salts is given by

$$E = \frac{RT}{nF} \log_e \frac{P}{p} \quad \ldots\ldots\ldots \quad (1)$$

where $R =$ the "gas constant," $T =$ absolute temperature, $F = 1$ Faraday and $n =$ valency of the metal ion.

Now if we connect together two such systems only differing in the concentration of ions, the *E.M.F.* of the cell (called a concentration cell) will be given by $E = E_1 - E_2$. Substituting E_1 and E_2 from the equation (1) and remembering $P_1 = P_2$ we have

$$E = \frac{RT}{nF}(-\log_e p_1 + \log_e p_2) = \frac{RT}{nF}\log_e \frac{p_2}{p_1} = \frac{RT}{nF}\log_e \frac{c_2}{c_1} \quad \ldots \quad (2)$$

since p is proportional to the concentration of ions, c.

Now a strip of platinum coated with platinum black dipped into a solution of $H^{\cdot}$ and saturated with hydrogen gas behaves just as the metal dipped into a solution of its ions. Therefore if we set up a concentration cell of two hydrogen electrodes and cH of one solution is known we can find the other from equation (2). If the known solution is normal, its cH is unity and equation (2) becomes

$$E = \frac{RT}{nF} \log_e \frac{1}{\text{cH}} = 0{\cdot}058 \log \frac{1}{\text{cH}}$$

substituting values for the constants and changing from natural to common logs. (Temperature = 18° C.)

Hence $$E = 0{\cdot}058\ \text{pH} \quad \ldots\ldots\ldots \quad (3)$$

In practice it is more convenient to use a calomel electrode than a normal hydrogen electrode. Correcting for the difference of potential of the electrode, equation (3) becomes

$$\text{pH} = \frac{E - 0{\cdot}25}{0{\cdot}058} \text{ at } 18^\circ \text{ C.}$$

(2) **Glass Electrode.** The hydrogen electrode method is difficult to apply to biological fluids containing carbonic acid, since the hydrogen gas displaces CO_2 from the solution and so changes its pH during measurement. This is avoided when a glass electrode is used. This method depends upon the fact that when solutions of different cH are separated by a thin partition of special glass a potential difference is established dependent upon their respective cH. Each solution is connected with a calomel electrode. If the cH of one solution is known the other can be calculated from

$$E = 0{\cdot}058\ (\text{pH}_x - \text{pH}_s) \text{ at } 18^\circ \text{ C.}$$

where pH_x = pH of the unknown and pH_s = pH of the known solution. This method can be readily applied to blood and tissues of which only very small amounts are required (about 0·5 c.c.).

The electrical methods are much more accurate than the colorimetric methods. The former are accurate to about 0·01 pH, the latter barely 0·1 pH. For the details of the measurement of *E.M.F.* in the electrical methods, see Refs. 5, 11, 12, 14.

CHAPTER III

WATER (2, 16, 17)

GENERAL, OSMOSIS, SURFACE TENSION AND ADSORPTION

While it is impossible to label any compound in the body as the most important, since there are many substances essential to its continued existence, there is no doubt that water is unrivalled in the number of essential functions it performs in living tissues. We are largely composed of water, and water predominates even in our solid food. With the exception of air, deprivation of water is more rapidly fatal than the removal of any other substance from our daily intake. Men have abstained from all food for two months and recovered, but have always taken water; if neither food nor drink is taken death ensues in a very short time. In the desert, deprivation of food and water is fatal in about 4 days; an Italian political prisoner is stated to have survived for 18 days. Death usually ensues when about 20% of the body water has been lost. In severe illness when all food is refused, water is usually readily taken. While the sensation of hunger is said not to persist long during starvation, there is always a sensation of thirst in water deprivation. In health we take water (in various forms) with as little thought as we take the air we breathe, while we worry about our calories and vitamins which can be dispensed with for a month without irrevocable consequences.

Water Content of the Tissues

Approximately two-thirds of the adult body consists of water. The water content of different tissues varies considerably from 10% in the dentine to 99% in cerebrospinal fluid. The figures for soft tissues (except fat) vary from 68% to 86%; the skeleton from 14% to 50%; fat contains from 6% to 20% of water. The values are very approximate, at least with some organs, because the water content varies very considerably from time to time. This is especially so in the muscles and skin which form the chief water storage tissues. A man of 11 stone (70 kg.) would contain nearly 47 litres of water, of which about 20 litres would be in the muscles and 10 in the skin ; blood (water content 79%) contains 4–5 litres of water. (The actual blood volume is by no means certain, so that this figure is very approximate.)

There is a tendency for young animals and actively metabolising tissues to have a greater water content than old animals and relatively inactive tissues. The water content of a baby is about 70%. The percentage water content of a mouse at different stages of development is given in the following table :—

WATER CONTENT OF MOUSE (v. Bezold)

State of Development	Water Content
Fœtus	87%
Newborn	83%
Eight days old	77%
Full grown	71%

The water content of different types of life vary very greatly. A lichen is probably one of the driest ; one species of jelly-fish contains 95·6% of water ; a tadpole contains about 90% water.

Water Content of Food

Apart from drinks, we take a large amount of water in our "solid" food. The percentages of water in various foodstuffs can be found (by difference) in Chap. XXXI. An ordinary meal weighing about 600 g. will contain from 50% to 60% (*i.e.*, 300–350 c.c.) of water ; such a meal is usually accompanied by drinks or beverages.

The amount of water consumed varies according to the temperature and type of food. In England our total water intake (food + drink) is normally between 2 and 3 litres ; in India and countries with similar climates it may be 13 litres.

The Water Intake and Output

In the adult in metabolic equilibrium the water intake must balance the water output. All factors concerned in the output of water are liable to variation ; the expired water from the lungs will be increased if respiration is increased; the water excreted by the kidney as urine will depend not only on the water ingested but also on the type of food, *e.g.*, a high protein diet leads to increased excretion owing to the diuretic action of the urea formed. By far the most variable factor is the water eliminated through the skin, for this is the chief method by which constant body temperature is maintained. Even when not visibly perspiring a man at rest loses about 18 g. water per hour (430 c.c. per day); a collier may lose as much as 7½ litres in a shift (8 hours) in the mine. A sedentary worker is usually stated to lose about 700 c.c. per day. These variations in output are balanced by changes in the volume of water ingested.

On the credit side, in addition to food and drink, we must consider the water actually formed in the body during oxidation of food. Under equilibrium conditions the whole of the food must be regarded as being oxidised to CO_2 and H_2O, or excreted, for even the protein used for tissue repair is balanced by the oxidation of an equivalent amount of broken-down tissue. The amount of hydrogen (other than water) excreted, *e.g.*, as urea, ammonia, etc., is relatively small and would not amount to more than 5 g. per day. Consequently practically the whole of the non-water hydrogen of the food must be considered as being converted into water. On an average diet about 450 c.c. of water would be formed in this way each day.* A rough balance sheet for a man under average conditions (light work) is given in the table.

WATER BALANCE SHEET (PER DAY)

Credit		Expenditure	
Drinks . . .	1,350 c.c.	Lungs . .	500 c.c.
Food . . .	900 c.c.	Skin . .	700 c.c.
Oxidation of food .	450 c.c.	Urine . .	1,400 c.c.
		Fæces . .	100 c.c.
	2,700 c.c.		2,700 c.c.

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* In hibernating animals all the water needed for metabolism is formed in this way, chiefly by oxidation of fat. The clothes-moth can live and produce normal eggs in a desiccator, its water being provided entirely by oxidation.

We have also a continuous excretion of water in the form of digestive juices from the body into the alimentary canal and subsequent reabsorption along with the water of food and drink. The amount of this internal circulation is from 7 to 10 litres per day (pp. 190–195). All of this water is reabsorbed into the body with that of food and drink, with the exception of about 100 c.c. excreted in fæces. The extent of the secretion of saliva is more readily appreciated when we have a " sore throat."

The Chemical Constitution of Water

The customary conception of water as H_2O with a molecular weight of 18 is certainly incorrect for water in the state in which we use it. If it is compared to the gas N_2O, it is indeed surprising that the simpler molecule H_2O is a liquid with relatively high boiling-point. H_2O probably only exists above 400° C. The old idea of water as a simple polymer of the type $(H_2O)_n$ is not in accord with modern physico-chemical evidence. The abnormal properties of water are ascribed to the arrangement of H_2O molecules into groups held together by dipole forces. (Water molecules have opposite " poles " on each molecule, so that molecules will tend to attract each other by their opposite " poles "). But, whatever its true formula, water has several very remarkable physical properties which make it pre-eminently suitable for its *rôle* in living tissues.

Biologically Important Properties of Water

Specific Heat. More heat is required to raise the temperature of 1 g. of water 1° C. than almost any other known solid or liquid. This means that heat liberated from reactions in the cell will cause the minimum rise in temperature.

Latent Heat of Evaporation. Not only is water an ideal heat buffer but also it has the highest known latent heat of evaporation. This means that the maximum cooling is obtained by evaporation of a given amount of water. Further, air can hold more water vapour for a given temperature and pressure than any other vapour, so that more water can evaporate than any other liquid.

These three maximal properties and the ease with which water is transported make it the ideal agent for the regulation of body temperature. Heat regulation by sweating is also very economical, since water is the most readily available of all liquids.

Solvent Power. Perhaps the most remarkable property of water is its power of dissolving substances. Water can form true solutions and colloidal solutions, but even that does not exhaust its solvent power, since insoluble substances can be dissolved by hydrotropic action of soluble substances (p. 47). In this way all the substances are brought into contact and chemical reactions are possible. The great solvent power of water is the more readily appreciated when the insolubility of most compounds of the body in other solvents is considered ; even in alcohol the majority are insoluble.

Dielectric Constant. The dielectric constant of pure water is higher than that of any liquid except hydrogen cyanide. This means that oppositely charged particles can co-exist in water. It is, therefore, a good ionising medium. This increases the possible range of chemical reactions.

Catalytic Action. Another remarkable property of water is its catalytic action. Whether due to its ionising power, readiness to form intermediate compounds or some other reason, water accelerates a very large number of chemical reactions. In the body all reactions proceed in the presence of water ; many reactions do not proceed in the absence of water, *e.g.*, neutralisation of acid and base, combination of oxygen and hydrogen. It has even been said that no chemical reaction proceeds in the *complete* absence of water.

Lubricating Action. Like all machines with moving parts, the body requires lubricants to prevent friction on rubbing surfaces, such as the joints, pleura, conjunctiva and peritoneum. Here again aqueous solutions, practically free from grease or oil (*i.e.*, lipides), provide a perfect lubrication for the joints and rubbing viscera over the span of man's life. The exact nature of synovial fluid and the other mucus-like lubricating secretions of the body is unknown.

PROPERTIES OF SOLUTIONS

Diffusion

A lump of sugar placed in a glass of water gradually disappears, and even if the liquid is kept perfectly still ultimately forms a homogeneous solution of sugar in water. This is due to energy possessed by all molecules, which keeps them in a state of perpetual motion or vibration. This spreading of solute molecules throughout the water molecules is called *diffusion*. Diffusion in liquids is a relatively slow process because the molecules are so crowded together that they are continually colliding and it may take a month or more for the lump of sugar to diffuse into water and form a homogeneous solution. If, however, the crowding is relieved so that molecules are able to move without continually colliding with solvent molecules, as in a gas, diffusion is rapid. Now in a liquid, not only are the molecules possessed of kinetic energy which causes the perpetual vibratory movement just referred to, but also they are subjected to an attractive force which is the gravitational force by which particles of matter attract each other. The former is responsible for the phenomenon of diffusion and osmosis, the latter surface tension.

Osmotic Pressure *

We have just seen that the kinetic energy of molecules causes them to diffuse in an attempt to form a homogeneous solution. This diffusion will take place against gravity. If we place a concentrated solution of lead nitrate at the bottom of a tall jar full of water a homogeneous solution of lead nitrate will be formed if we leave the jar undisturbed for a few months. This tendency to form a homogeneous mixture of solute and solvent molecules is a strong one, as we can see if we hinder the process by preventing the solute molecules diffusing. If we cover a concentrated sugar solution with a layer of water the two layers will mix and ultimately form a uniform solution. But if the two layers are separated by a membrane which permits free passage to water but not solute molecules (a *semi-permeable membrane*) diffusion of solute molecules will be prevented. Water molecules, however, are not prevented from diffusing and they continually pass into and out of the sugar solution. Since, however, in a given time relatively more water molecules collide with (and pass through) the membrane on the water side than on the sugar side which is bombarded by both solute and water molecules, more water will pass into the sugar solution than out, and this process will go on until homogeneity is achieved unless opposed by some other force. If the experiment is conducted in an apparatus such as Fig. 1, the *volume* of sugar solution in A will increase as in Fig. 2 until the hydrostatic pressure in A forces the water molecules out at the same rate as they enter from B, *i.e.*, when the hydrostatic pressure balances the diffusing force.

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* The kinetic picture of osmotic pressure presented here is not in accord with modern views. Actually there is no satisfactory explanation of osmotic pressure.

If, instead of letting the volume of solution A increase, we enclose the space and connect it with a mercury manometer as in Fig. 3 a *pressure* will develop, since the volume cannot increase, and this pressure will be a measure of the diffusing force or effort of the molecules to achieve homogeneity. The pressure applied to the sugar solution which just stops the diffusion is called the *osmotic pressure* or *osmotic water attraction* of the sugar solution and the passage of water due to this attraction is called *osmosis*. It can be shown by experiment and by thermodynamical argument that the osmotic pressure of a very dilute solution is the same as that which the solute molecules alone would exert if they existed as a gas in the same volume as that of the solution (*i.e.*, 1 g. molecule dissolved in 22·4 litres would exert an osmotic pressure of 1 atmosphere, since 1 g. molecule of a gas at N.T.P. occupies 22·4 litres). Just as we increase the pressure of a gas by diminishing its volume, so we increase osmotic pressure by diminishing the volume of solvent in which the solute is dissolved, *i.e.*, by increasing the concentration of the solution. We shall, therefore, have an osmotic difference of pressure set up whenever two liquids of different osmotic pressure (*i.e.*, different molecular concentration) are separated by a semi-permeable membrane. Fig. 2 or 3 would apply equally well if A were a strong and B a weak solution of the same substance. Both strong and weak solutions would show an osmotic pressure

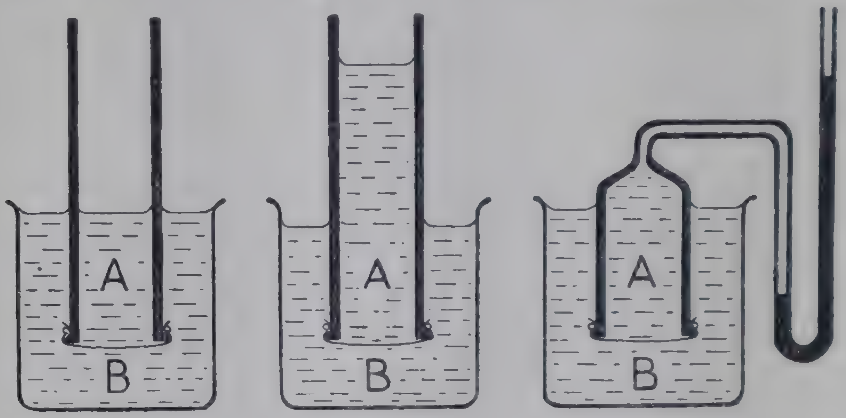


FIG. 1. FIG. 2. FIG. 3.

A. Sugar solution. B. Water or more dilute sugar solution.

against water, but that of the strong solution would be greater. In the absence of inhibiting forces, *if two solutions of different osmotic pressure are separated by a semi-permeable membrane, water will pass from the solution of low osmotic pressure to that of high osmotic pressure until the osmotic pressures on the two sides of the membrane are the same.* This applies regardless of the chemical nature of the solutes in either solution and applies to mixtures (in which the total osmotic pressure of the solution is the sum of the osmotic pressures of the individual constituents), provided the membrane is only permeable to water.

It should be noted that osmotic pressure is only a *potential* pressure and not manifest until the solution is placed in contact with another solution with a membrane interposed. A 6% cane sugar solution in an apparatus such as Fig. 3 (an osmometer) exerts a pressure of 4 atmospheres; but this pressure is not exerted on the walls of the bottle in which the solution is stored.

Osmotic pressure is only permanent if the membrane is truly semi-permeable, *i.e.*, if it stops all solute molecules and only passes solvent molecules. In practice this is rarely the case, except in specially constructed osmometers. For experimental purposes, such as dialysis (p. 39), the vessel A in Fig. 2 or 3 takes the form of a collodion or parchment bag. Such a membrane is permeable to small molecules but not to large. If the bag were filled with a solution of a dye with small molecules such as phenol red and placed in contact with water, water will pass into the dye but at the same time water plus dye will pass out from the bag. Since water molecules are smaller than the dye molecules, water will pass into the bag more quickly than water plus dye will leave it. Therefore an osmotic pressure will be developed, but it will only be small and *transient* because the membrane is permeable to both water and dye. If, however, the bag were filled with a dye such as Chicago blue, which has molecules too big to pass through a collodion membrane, the dye would not pass out into the water, but water would pass into the dye and cause a *permanent* pressure difference. If instead of water we surrounded the bag with a solution of NaCl of greater osmotic pressure than the blue dye, water would at first pass out of the bag faster than it went in, but at the same time NaCl would pass into the bag, since collodion is permeable to it, and final equilibrium would be obtained when the osmotic pressure of the salt solution outside the bag equalled the osmotic pressure of salt inside, so that the ultimate permanent osmotic pressure difference would be that of the dye alone. This is a point of some significance in the absorption of food (p. 208), since cell membranes are not truly semi-permeable.

In the foregoing we have referred to osmotic pressure as being dependent on the *number* of molecules of solute relative to solvent molecules. It must be clear that the size of the molecules is of no significance. A solution of urea (M.W. 60) of 60 g. per litre has the same osmotic pressure as a solution of cane sugar (M.W. 342) of 342 g. per litre, because these two solutions contain the *same number* of molecules per litre. Actually it is more correct to say that the osmotic pressure is dependent upon the number of solute particles. In salts which ionise it is the number of ions plus molecules which count, so that fully ionised NaCl has twice the osmotic pressure it would have as judged by the number of molecules. Other substances, such as soaps, form molecular aggregates, so that their solutions have lower osmotic pressures than would be expected.

Some Applications of Osmosis. The purgative action of Epsom ($MgSO_4$) or Glauber's (Na_2SO_4) salts was at one time supposed to be purely an osmotic phenomenon. A strong solution of a salt in the intestine either prevented absorption of water or even withdrew water from the body into the intestine, thus greatly diluting the intestinal contents. This view is probably not correct; the purgative action of salts is more complex. The pain associated with contact of sugar with exposed nerves of teeth is probably due to a transient osmotic withdrawal of water from the exposed area by strong sugar solution. The smarting of a cut on the skin exposed to salt has probably a similar explanation.

We shall see later several applications of osmosis in the process of absorption (p. 207) and hæmolysis (p. 163). The cells in the body are *isotonic* (in osmotic equilibrium) with the tissue fluids and blood plasma. An appreciable osmotic dilution of plasma would create a dangerous hydrostatic pressure in the red cells and tissue cells which would take in water to achieve osmotic equilibrium. This does not occur in the body because water or salts (chiefly NaCl) are excreted by the kidney so as to keep the blood isotonic with the cells. Osmosis is of great importance in the process of urine secretion. A clinical application of osmotic force is the injection of hypertonic solutions of salts such as magnesium sulphate to reduce the volume of the brain or lower the pressure of cerebrospinal fluid. Water is withdrawn from the brain osmotically and may be removed from other tissues in the same way.

Before leaving this subject some terms commonly used in Biochemistry and Physiology may be defined more clearly.

Two solutions are *isotonic* when they are in osmotic equilibrium when separated from each other by a membrane. If both solutions contain only substances which pass freely through the membrane the solutions are also *isosmotic*, that is, they have the same osmotic pressure. If, however, one solution contains substances which cannot pass the membrane, the solutions, *although they may be isotonic, are not necessarily isosmotic*. Thus a man's blood is isosmotic with about 0·945% NaCl (see p. 152), but it is isotonic with a slightly lower concentration of NaCl, as a small part of the osmotic pressure is contributed by the non-diffusible plasma proteins. The term isotonic is, however, frequently used in both senses. A *hypotonic* solution is one more dilute than isotonic and a *hypertonic* solution one more concentrated.

Since the osmotic pressure is proportional to the number of molecules (or molecules plus ions) in solution we can determine osmotic pressure by any method giving the number of molecules in solution. Since direct determination is technically difficult, osmotic pressure is usually determined indirectly by measuring the depression of freezing-point or of vapour pressure of the solution. A very accurate method of determining the latter has been devised by A. V. Hill.

Surface Tension

Now let us examine the attractive force (p. 23) existing between the molecules of a liquid. Within the liquid a molecule will be attracted equally in all directions by other molecules (as at **A**, Fig. 4), and hence can move with equal freedom in any direction. But a molecule at the surface is only attracted by molecules within the liquid (as at **B**), so that it tends to be pulled inwards and its freedom of motion is restricted. This causes the surface of a liquid to pull itself together tending to occupy the least possible area, and a layer of greater density in a state of tension is formed. This surface film has the properties of an elastic skin and is resistant to rupture, as can be seen by the experiment of floating a needle on water. Other manifestations of this surface tension are formation of drops by liquids falling through air, *e.g.*, raindrops or drops from a tap, or through liquids in which the drop is insoluble, the rise of liquid in a capillary tube, formation of a meniscus at the surface of liquids in vessels ; the motion of camphor " boats " is due to uneven solution of camphor in water causing uneven lowering of the surface tension of the water and, therefore, uneven forces acting upon the " boat " so that it moves.

A great part of the energy required to convert a liquid into a gas (*Latent Heat of Evaporation*) is required to overcome surface tension and drag the molecules free from the surface of the liquid. In the foregoing we have been considering the condition of molecules at a liquid-gas junction. The same applies to all junctions between immiscible phases, *e.g.*, liquid-liquid, liquid-solid junctions. Such junctions are usually called *interfaces* and the strain or tension at interfaces is called the *interfacial tension.* We shall see later that interfacial tension is of great biochemical importance, especially in the process of adsorption.

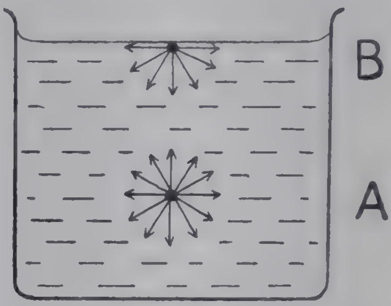


FIG. 4.

Effect of Solutes on Surface Tension and Surface Energy. The effect of solutes on surface tension cannot be predicted. Most inorganic salts slightly raise, although potassium permanganate lowers, the surface tension of water (in air). Organic substances usually lower surface tension ; soaps and bile salts are particularly effective in this respect. In liquid-liquid and solid-liquid systems dissolved substances generally lower interfacial tension.

It is a fundamental physical law that if any change favours the reduction of free energy in a system, that change tends to take place. Now *surface energy*, which is the product of the surface tension times the surface area, is a form of free energy. Therefore there is always a tendency for reduction of surface energy. This is most readily seen in the behaviour of a soap film stretched across the wide end of a funnel held stem upwards as at **A**, Fig. 5. The film will only remain at A if we prevent the reduction of free energy by closing the stem of the funnel so that air cannot escape. If the end is free, the free energy of the system is reduced by reduction of the surface area by the film moving up to the point of smallest area **B**. Here surface energy has been reduced by *reduction of surface area*; it may also be reduced by *diminishing surface tension.* This is seen in the behaviour of surface tension lowering substances when added to a solution. Since substances which lower surface tension would be most effective if their molecules were concentrated at the surface, such substances tend to concentrate at the surface of the liquid; conversely, surface tension raising substances tend to move away from the interface. The concentration of surface tension lowering substances is often visible to the naked eye. When an aqueous solution of a dye such as bromthymol blue is shaken up with carbon tetrachloride, the interfacial concentration of the dye is readily seen as the droplets coalesce; a protein layer is often obtained at the interface when a protein solution is shaken up with ether. Lipides and proteins, which are both effective in lowering surface tension, are found concentrated in the cell wall (see also Adsorption, p. 31).

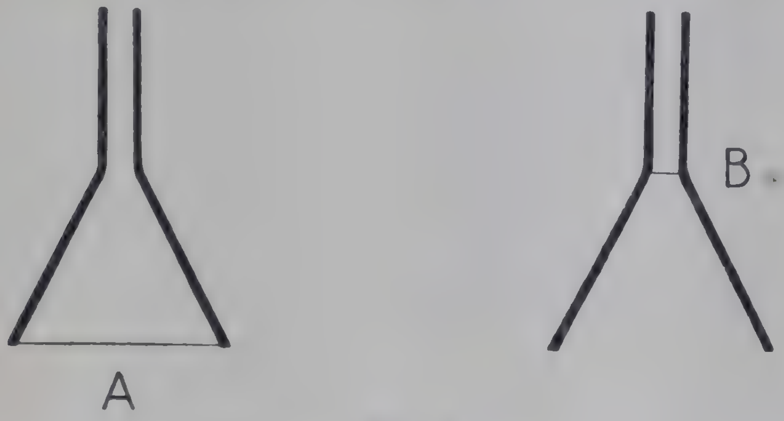


FIG. 5.

A mixture of equal volumes of water and chloroform in a test tube has a relatively small interfacial area. If the mixture is shaken droplets are formed which *in toto* have a very much greater interfacial area (p. 38). As soon as shaking ceases these droplets coalesce to form two layers so as to reduce the free energy of the system (by reducing surface area). Now if a surface tension lowering substance such as bile salts or soap be added and the mixture shaken, it will take much longer for the droplets to coalesce because the free energy of the system has already been largely reduced by the lowering of surface tension due to the bile salts or soap. In this way these substances assist emulsification. Bile salts are of value in this respect in the process of digestion. Soluble proteins or gums are often used for this purpose in the manufacture of emulsions.

A practical application of lowering of surface tension is Hay's Test for bile salts in urine. The surface " skin " on normal urine is sufficiently dense to prevent fine particles of sulphur sprinkled on the surface from penetrating the " skin " and sinking to the bottom ; the presence of small amounts of bile salts, as in the urine of certain types of jaundice, so lowers the surface tension that the " skin " can no longer support sulphur particles and they sink to the bottom of the test tube.

Surface tension can be measured in several ways, such as direct measurement of the force required to pull a platinum ring off the surface of a liquid, the rate of drop formation in a stalagmometer, or the height to which a liquid rises in a capillary tube. The result is expressed in dynes per centimetre. Some typical values are given in the table.

INTERFACIAL TENSIONS IN DYNES PER CM. AT 20° C.

Substance in Contact with	Air	Water	Mercury
Mercury	487·0*	375·0	—
Water	72·7	—	375
Aniline	42·9	5·8	341
Toluene	28·4	36·1†	359
Chloroform	27·1	32·8	—
Ethyl alcohol	22·3	—	364
Ethyl ether	17·0	10·7	379

* At 15° C. † At 25° C.

Note the high value for mercury ; this is in agreement with the readiness with which mercury forms spherical globules when dropped on the bench ; water only does this on a dusty or greasy bench, alcohol practically never. Note the low value for ether and the way in which it pours from a bottle.

Adsorption

The interfacial concentration of dissolved substances, as described on p. 29, may be of considerable magnitude if the interfacial area is sufficiently large, as, for example, when a fine powder is shaken up in a solution. If a suitable dye solution is shaken up with animal charcoal and filtered, the filtrate may be colourless, because the interfacial concentration of the dye on the charcoal has removed it all from solution. This process of taking up substances from solution on surfaces is called *adsorption.* Adsorption phenomena are particularly marked in colloidal solutions which have extremely large interfacial areas. Adsorption is not, however, a purely interfacial phenomenon. Dyes, for example, are adsorbed by charcoal to a much greater extent than would be expected if the process were only a question of reduction of surface energy and, furthermore, they are not adsorbed by all samples of charcoal. The adsorption of arsenic by colloidal iron, formerly the basis of a method of treatment of acute arsenic poisoning, could also be explained on electrical grounds as the neutralisation of electric charges, since colloidal iron particles carry a positive charge and arsenic a negative (arsenic is usually taken in the form of arsenious acid). Most finely divided solids in solution carry electric charges. Charcoal acquires a negative charge when suspended in water, and the efficiency of adsorption of dyes by charcoal is probably partly due to electrical forces. Failure of adsorption might be ascribed to similarity of electric charges causing repulsion of the dissolved substance from the particles. The electric charge of the particles may be considerably altered by the presence of salts, acids or alkalis. Another complicating factor is the possibility of chemical reaction between the particles and adsorbed substance. Adsorption is a complex process which has not yet been completely explained. Biologically, adsorption is probably primarily a surface tension phenomenon owing to the enormous interfacial areas existing in colloidal solutions, but superimposed electrical and chemical reactions almost certainly occur.

Adsorption is probably the secret of many of the remarkable reactions which occur in the body, for adsorption can assist chemical reactions by bringing adsorbed molecules into close contact, and so facilitate chemical change. The adsorbing surface acts, in fact, like a catalyst; adsorption is undoubtedly of considerable importance in the action of enzymes (which are colloids with large interfacial areas). In the presence of an adsorbing surface reactions take place which cannot be detected in their absence. Amino-acids in water do not react with oxygen; if, however, active charcoal is present oxidation occurs.

Adsorption is often reversible, indicating that probably no chemical reaction occurs. Methylene blue adsorbed on charcoal can be extracted from the charcoal by suitable solvents (alcohol, acetone). Substances such as enzymes, which are easily destroyed by chemical treatment, are frequently purified by adsorption on a suitable medium such as aluminium hydroxide and subsequent extraction by a suitable solution, *e.g.*, one of different pH or salt content. Recovery of an adsorbed substance in this way is called *elution.*

EXAMPLES OF ADSORPTION AND SURFACE PHENOMENA

(1) **Charcoal.** Suitably prepared charcoal is very active in adsorbing not only substances in solution but gases. It finds application in the decolorisation of coloured solutions, the clearing of cloudy solutions and in purification by adsorption and elution, *e.g.*, enzymes and insulin. The most important application in the adsorption of gases is in the preparation of gas masks. Most poisonous gases, but not carbon monoxide, are adsorbed from the air by active charcoal.

(2) **Sewage Treatment.** Impure water draining from cultivated and manured land and sewage water is rendered completely harmless by passing through soil or charcoal beds in which impurities are adsorbed at the surface of soil colloids or the charcoal. Drinking water can be safely prepared in this way.

(3) **Action of Enzymes.** Adsorption is of considerable importance in assisting enzyme action by bringing the reactants into close contact.

(4) **Dyeing and Staining.** In the dyeing of textiles and staining of histological sections the chief factor is more often adsorption than chemical combination.

(5) **The combination of Toxin and Antitoxin** is probably largely an adsorption process.

(6) **Adsorption Compounds.** Many loose compounds are formed in the body which appear to be adsorption rather than chemical compounds. Lecithin forms such compounds with proteins, *e.g.*, lecithovitellin in egg yolk and similar compounds in brain. These compounds are insoluble in ether (lecithin is soluble) but are separated by extraction with other solvents, *e.g.*, alcohol. Compounds of this type are probably very abundant in protoplasm. The blue substance formed from starch by iodine is an adsorption compound.

(7) **Surface Films.** Examples of visible interfacial concentrations of proteins and dyes have already been given. Such a concentration of protein may cause its coagulation. This is most clearly seen in the froth produced by beating egg-white. Protein becomes concentrated on the surface films (liquid-air interface) and coagulated, for after the froth has settled on standing it cannot be beaten up again to a froth of anywhere near the same size.

(8) **Clearing of Soups and Media.** Clear soups are not obtained in the kitchen by fine filtration. The constituents causing cloudiness are removed by addition and coagulation of egg-white, on the particles of which the unwanted constituents are adsorbed. The clear soup is then separated by decantation, or straining. A similar process is used in clearing broths for culture media.

(9) The following laboratory experiment is of interest. A solution of an indicator in a buffer of suitable pH is added to an equal volume of carbon tetrachloride. If the indicator is bromthymol blue and the aqueous solution green, the colour changes to yellow when the solution is shaken vigorously. This is not due to an optical effect, for a solution made up to match the green with non-indicator dyes remains green on shaking. The change is even more striking with thymol blue (acid range) when the colour changes from orange to red on shaking. In the alkaline range a blue solution of thymol blue gives a greenish grey colour on shaking. These changes are completely reversible, the original colour returning when shaking ceases. Interfacial concentration of the dyes is well shown as the two layers separate. Whether the explanation be a depression of ionisation of the dye adsorbed, and so concentrated, on the CCl_4 droplets or a selective adsorption of $H^{\cdot}$, there is no doubt that a chemical change (in that the colour of the indicator changes) has occurred through changing the interfacial area. The apparent pH change is a lowering by nearly one unit of pH. The experiment illustrates the possibilities of chemical change through alteration in interfacial area.

Arrangement of Molecules at an Interface (2)

Solute molecules accumulated at an interface tend to arrange themselves in a definite pattern if the molecules are unsymmetrical, whereas within the solution the arrangement is haphazard, since forces act upon the molecules equally in every direction. The term unsymmetrical is used here in the sense that the molecules have water attracting (*polar*) groups, *e.g.*, $-COOH$, $-OH$, $-NH_2$, unsymmetrically disposed as in a fatty acid or alcohol, in contrast with symmetrical molecules such as a paraffin, benzene, a sugar, or water itself. These unsymmetrical molecules align themselves so that the polar groups are directed towards the water and the non-polar groups at the other end of the molecule away from it (*orientation*). If oleic acid is spread over the surface of the water in a film 1 molecule thick (this can be done by dropping a solution of oleic acid in a volatile solvent on a large surface of water and letting the solvent evaporate) the molecules orient so that the carboxyl groups are in the water and methyl groups in the air, so that there is, in effect, a surface film of paraffin hydrocarbon. This can be represented as in Fig. 6.

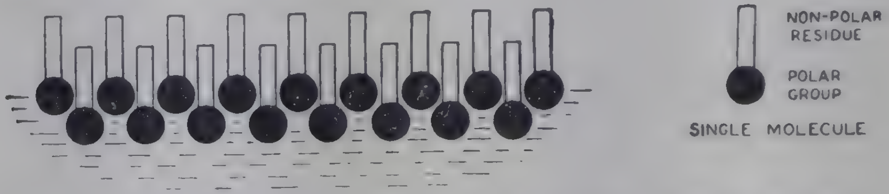


Fig. 6. Interfacial orientation of oleic acid. (After Harkins.)

At an oil-water interface the orientation would be especially favoured, since the oil would attract the non-polar groups in addition to the water attracting the polar groups.

In the cell, which contains both water and lipides, it is likely that some cell constituents are oriented in this way ; it is even conceivable that the cell membrane is essentially adsorbed oriented molecules. Orientation may be an appreciable factor in many adsorption and enzyme reactions, since in this way one part of a molecule could be preferentially presented to a reacting substance.

CHAPTER IV

WATER (*continued*) (2)

THE COLLOIDAL STATE, HYDROTROPY AND HEAVY WATER

About 90% of the organic matter of living tissues is present in the colloidal state, so that it is important that the meaning of this term should be clear. Graham in 1861 was the first to distinguish two types of solutions: *crystalloidal*, which diffused through a parchment or animal membrane, and *colloidal*, which did not. To-day we can only improve on Graham's definition by quoting arbitrarily fixed measurements for the sizes of molecules which form crystalloidal or colloidal solutions. The difference is really only one of the size of the solute particles and is unrelated to their chemical nature. If particles are of the size of small molecules like sugar, urea or NaCl, they form a crystalloidal or a true solution; in such a solution the difference in size of solute and solvent molecules is relatively small, so that the solution can be called homogeneous. If the particles are large compared to the solvent molecules, we have a heterogeneous system, and if the particles do not separate when the "mixture" is allowed to stand, this is called a colloidal solution. Let us consider some stages in the disintegration of a piece of gold.

(1) *Lump of Gold.* If the piece of gold is placed in water we have two phases and a heterogeneous mixture.

(2) *Gold Dust.* If the piece of gold is filed down to fine dust and suspended in water we still have two phases, even though a microscope be needed to see the smallest particle. The particles are enormous compared to water molecules, and ultimately settle to the bottom of the vessel, leaving the supernatant liquid free from gold.

(3) *Colloidal Gold.* If an electric arc is formed between two pieces of gold under water, we can disintegrate the metal into

particles so fine that they cannot be seen even by the ordinary microscope. Their presence can, however, be indicated by the ultramicroscope, which consists of an ordinary microscope arranged so that the solution under examination can be illuminated in a direction at right angles to the optical axis of the microscope by an intense beam of light from an arc lamp. Under these conditions the light reflected by the particles can be seen as bright spots; the solution somewhat resembles the astronomer's "Milky Way" in appearance, except that the particles are always moving, for a reason which will be explained later. Now these particles never settle and gold is always present in the solution (which is actually coloured either red or blue). This is a colloidal solution of gold, but it is still a heterogeneous system, since the particles are very large compared with water molecules.

(4) *Ionic Gold.* By dissolving gold in *aqua regia* we can disintegrate it into the finest possible particles, namely, gold ions. These are invisible even to the ultramicroscope, and their size is of the same order as that of water molecules, so that we have a homogeneous system and true solution.

Some very interesting properties are associated with colloidal solutions, and by measurement of the size of the particles in solutions with these properties the range of particles which form colloidal solutions has been defined. Obviously colloidal solutions gradually merge into true solutions at one end and suspensions at the other end of the range. Very small particles have been arbitrarily divided into three groups according to their diameters. First let us define the small units of length. One thousandth of a millimetre is called a *micron* and symbolised μ. A thousandth of a micron is a *millimicron*, symbolised $m\mu$. The three groups of small particles are given in the table :—

Group	Name	Size	Properties
1	Microns .	Over 100 $m\mu$.	Invisible to naked eye, but visible under microscope; ultimately separate from solution.
2	Submicrons .	Between 1 $m\mu$ and 100 $m\mu$.	Only visible under ultramicroscope; permanent solution.
3	Amicrons .	Below 1 $m\mu$.	Completely invisible; permanent solution.

Only amicrons give true solutions. *Submicrons give colloidal solutions.* Microns give suspensions or emulsions which gradually separate. Examples of each class with particle or molecular diameters are given in the following table :—

	Examples	
Microns	Red blood corpuscles (suspension)	8 μ
	Globules of fat in milk (emulsion)	2 to 10 μ
Submicrons	Colloidal gold particles	2 to 20 mμ
	Starch	5 mμ
Amicrons	Cane sugar	0·7 mμ
	Sodium chloride	0·26 mμ
	Hydrogen	0·1 mμ

Some substances, even soluble substances of small molecular size, may form either colloidal or true solutions under differing conditions. In water NaCl forms a true solution ; in benzene it forms a colloidal solution. Soaps form colloidal solutions in water and true solutions in alcohol. These colloidal solutions are formed because small molecules aggregate to particles of submicron size. These aggregates are called *micelles.* Other substances have such large molecules that they are already submicrons and can never form true solutions, *e.g.*, substances of high molecular weight like polysaccharides and proteins.

In colloidal solutions the two phases are usually distinguished as the *continuous phase* or *dispersion medium* (the solvent) and the *discontinuous* or *disperse phase* (the suspended particles).

General Properties of Colloidal Solutions

Brownian Movement. The appearance of colloidal solutions under the ultramicroscope, described on p. 36, is observed in all living cells which can be examined in this way. The continuous motion of the particles is known as *Brownian Movement* and is observed in all colloidal solutions and in many micron suspensions, such as those of fine clay or gamboge.

If the reader has not observed Brownian movement he can easily do so by placing a drop of Indian ink (made from carbon, not dyes) on a slide and examining it under an ordinary microscope by transmitted light. The movement here is relatively slow owing to the large particle size ; in the finer colloidal solutions the motion is more rapid.

This Brownian movement partly (see p. 43) accounts for the permanency of colloidal solutions, for the particles are kept in movement by continuous buffeting by the solvent molecules, which are themselves always in motion. It might be described as a perpetual game of molecular billiards. The rate of Brownian movement depends on the size of the particles, the smaller particles obviously being more easily moved than the big ones. When the particles are too large to be moved easily on impact with a solvent molecule, they gradually sink under the influence of gravity. Brownian movement is quite haphazard. The actual paths of several particles have been mapped from cinematograph films of rubber latex and show jerky movements in a straight line with sudden irregular changes of direction.

Interfacial Phenomena. Many of the peculiar properties of colloidal solutions are due to the enormous interfacial area between the continuous and discontinuous phase. To give an idea of the interfacial area the table below gives the surface area of a sphere 1 cm. in diameter and the areas obtained by subdividing it in spheres of diameter 10 μ and 10 mμ.

Diameter of Spheres	Surface Area	Increase in Area
1 cm.	3·14 sq. cm.	
10 μ	3,140 sq. cm.	× 1,000
10 mμ	314 sq. m.	× 1,000,000

If this book were subdivided into spheres of 10 mμ diameter, the surface area would be approximately 130 acres.

A consequence of the enormous interfacial area of colloidal solutions is that adsorption is particularly favoured. This has already been discussed (p. 31). Some colloidal particles adsorb large amounts of water (p. 43).

Osmotic Pressure of Colloidal Solutions. Owing to the great size of colloidal particles relative to solvent molecules, there are only a few colloidal particles relative to solvent molecules in a given solution. Since the osmotic pressure of a solution is proportional to the *number* and not size of dissolved particles, *colloidal solutions have a low osmotic pressure.* The proteins of serum which are present to the extent of about 7% to 8% only exert an osmotic pressure of about 30 mm. Hg, whereas the crystalloids of serum ($\equiv$ 0·9% NaCl) have an osmotic pressure of about 6·8 atmospheres (= 5,200 mm. Hg) ; 6% cane sugar has an osmotic pressure of about 3,000 mm. Hg. Soap solutions have a small osmotic pressure because the soap molecules aggregate to form micelles of colloidal dimensions.

Although the osmotic pressure of colloidal solutions is very small, it may, nevertheless, be of considerable biological importance in providing the driving force for the passage of water and other substances through cell membranes (p. 159).

Dialysis. The large size of colloidal particles makes it easy to find membranes which are permeable to water and salts but impermeable to colloids. It has already been mentioned that Graham based his original distinction of solutions on their ability to pass membranes. The process of separation of crystalloids from colloids by diffusion through a membrane by osmotic force is called *dialysis*. The most usual membranes used to-day for dialysis are various grades of collodion (made from solutions of cellulose—nitrates or acetates—in solvents such as alcohol and ether or acetic acid) or cellophane ; parchment is sometimes used. Dialysis is especially useful for removing salts from, say, proteins after precipitation by " salting out." The precipitate with a little water is placed in a collodion bag and immersed in water as in Fig. 3 (p. 24). The passage of salt into the water is, of course, accelerated by keeping the salt content of the water low, *i.e.*, by having running water. Provision must be made for a big volume (or pressure) increase inside the bag in the early stages of dialysis, since it is a strongly hypertonic solution and water molecules will pass in more quickly than salt molecules pass out. But the pressure will only be transient if the water outside is frequently changed and the final volume (or pressure) inside the bag may be scarcely more than the original.

The process of dialysis must not be confused with the process of ultrafiltration, which may attain the same end and use the same membrane. Dialysis could be described as filtration through a membrane by *the agency of osmotic force only*. **Ordinary filtration** is attained by the force of *gravity or pressure*, positive or negative, through very porous membranes such as filter paper, which passes all molecules, crystalloid and colloid. In **ultrafiltration a** liquid is forced by the application of *very great pressure* through a membrane similar to those used in dialysis, and in this way large and small molecules may be separated.

Dialysis of electrolytes can be accelerated by passing an electric current through the solution. A cell consisting of three compartments separated by membranes is used ; the colloidal solution is placed in the centre compartment and the electrodes in the outer ones. Positive ions are attracted to the cathode and negative ions to the anode. This process is called **electrodialysis.**

The Donnan Equilibrium (13)

The diffusion of ions through a membrane may give rise to some very interesting results if the membrane is not permeable to all the ions, *e.g.*, if one of the ions is a colloid or a colloidal micelle. If the membrane is freely permeable, equilibrium between a solution of NaCl and KNO_3 would be attained when all four ions were equally distributed on each side of the membrane. When, however, the membrane is impermeable to one of the ions very different conditions prevail. Let us take the simplest case of solutions of NaCl and NaR (where R′ is an ion too large to penetrate the membrane) separated by a membrane. In all these examples complete dissociation of the compounds is assumed. The initial state can be represented :—

$$\begin{array}{c|c} \mathrm{Na}^{\cdot} & \mathrm{Na}^{\cdot} \\ \mathrm{R}' & \mathrm{Cl}' \\ (1) & (2) \end{array}$$

Since NaCl can diffuse from compartment (2) but not NaR from (1) equilibrium will be represented :—

$$\begin{array}{c|c} \mathrm{Na}^{\cdot} & \mathrm{Na}^{\cdot} \\ \mathrm{R}' & \mathrm{Cl}' \\ \mathrm{Cl}' & \\ (1) & (2) \end{array}$$

By application of thermodynamical principles it can be proved that the product of the concentrations of Na and Cl ions in compartment (1) is, at equilibrium, equal to the product of the concentrations of Na and Cl ions in (2).

$$[\mathrm{Na}^{\cdot}]_{(1)} \times [\mathrm{Cl}']_{(1)} = [\mathrm{Na}^{\cdot}]_{(2)} \times [\mathrm{Cl}']_{(2)} \quad . \quad . \quad . \quad . \quad . \quad (a)$$

It will, perhaps, be clearer if we express the molar ion concentrations as c_1 and c_2, and assume that x molecules of NaCl diffuse from (2) to (1). This could be represented :—

	Initial State				*Equilibrium*				
Ions .	Na˙	R′	Na˙	Cl′	Na˙	R′	Cl′	Na˙	Cl′
Concentrations	c_1,	c_1	c_2,	c_2	$(c_1 + x)$,	c_1,	x	$(c_2 - x)$,	$(c_2 - x)$

Applying equation (a), $(c_1 + x)x = (c_2 - x)^2$

$$\text{Hence } x = \frac{(c_2)^2}{c_1 + 2c_2}$$

From this the following numerical examples can be derived :—

Initial State				*Equilibrium*				
c_1,	c_1,	c_2,	c_2,	$(c_1 + x)$,	c_1,	x,	$(c_2 - x)$,	$(c_2 - x$'
100	100	100	100	133·3	100	33·3	66·6	66·6
100	100	30	30	105·6	100	5·6	24·4	24·4
100	100	1	1	100·01	100	0·01	0·99	0·99
10	10	100	100	57·6	10	47·6	52·4	52·4

Note that although the membrane is freely permeable to NaCl, a relatively high concentration of NaR practically inhibits diffusion from (2) to (1). Further, if NaCl be added to (1) when the concentration of NaR is high, NaCl will be " excreted " from (1) to (2).

Now let us consider four different ions, *e.g.*, KCl and NaR.

	Initial State				*Equilibrium*						
Ions .	Na˙	R′	K˙	Cl′	Na˙	R′	K˙	Cl′	K˙	Cl′	Na˙
Concentrations .	c_1,	c_1	c_2,	c_2	$(c_1 - z)$,	c_1,	x,	y	$(c_2 - x)$,	$(c_2 - y)$,	z
Numerical examples	100	100	100	100	66·6	100	66·6	33·3	33·3	66·6	33·3
	100	100	10	10	91·7	100	9·2	0·8	0·8	9·2	8·3

The numerical values are calculated from

$$x = \frac{(c_1 + c_2)c_2}{c_1 + 2c_2}, \quad y = \frac{(c_2)^2}{c_1 + 2c_2}, \quad z = x - y,$$

derived as before. Note in this case when the concentration of NaR is relatively high, Na is " excreted " to (2) and K is " selectively absorbed " into (1), and that this differentiation of the two ions isnot due to any peculiarity (or " selective permeability ") of the membrane.

Now let us consider the possibility of interaction between the salt NaR and water through a membrane. This can be represented :—

Initial State		*Equilibrium*	
$Na^{\cdot}$	$H^{\cdot}$	$Na^{\cdot}$	$Na^{\cdot}$
R'	OH'	R'	OH'
		$H^{\cdot}$	

The concentration, x, of H or OH ions at equilibrium is given by $x = \sqrt[3]{c_1 K_w}$, where K_w is the dissociation constant of water. Note that here we have a change of pH, the NaR side becoming acid and alkali being "excreted" across the membrane. The reverse change is possible with a salt RCl thus :—

Initial State		*Equilibrium*	
$R^{\cdot}$	$H^{\cdot}$	$R^{\cdot}$	$H^{\cdot}$
Cl'	OH'	Cl'	Cl'
		OH'	

Here acid is "excreted" across the membrane. Donnan points out that it is possible to attain in this way a concentration of H ions as great as that of gastric juice.

These theoretical speculations have been realised experimentally under appropriate conditions. Such conditions are, of course, far simpler than those which would prevail in living cells, where there would be several ions of each type and where complete dissociation could not be assumed. Nevertheless, the possibilities outlined above serve to show that many of the phenomena, which, to hide our ignorance, we have ascribed vaguely to selective absorption or selective membrane permeability, are very probably the outcome of very complex membrane equilibria of the type enunciated by Donnan. It must be remembered that in the body equilibrium may never be established owing to the removal of "excreted" ions in other reactions. This would, of course, accelerate the "excretion."

Emulsoids and Suspensoids

There are two types of colloidal solution, *emulsoids* and *suspensoids*. They have conspicuous differences in properties.

Suspensoids. The surface tension and viscosity of suspensoids are nearly the same as those of the solvent. The suspensoid particles carry a definite electric charge and are very easily precipitated by addition of small amounts of salts owing to its neutralisation. This charge probably determines the stability of the suspensoid, for similarly charged particles repel one another and do not tend to aggregate. Once precipitated, a suspensoid is not ordinarily brought back into colloidal solution again. They are therefore called *hydrophobe* or *lyophobe* colloids (water or solvent-fearing colloids).

Emulsoids. These colloidal solutions are relatively very stable and not easily precipitated by salts ; when precipitated, they are usually easily redissolved to form a colloidal solution, hence being called *hydrophile* or *lyophile* (water or solvent-loving) colloids. They have a lower surface tension and much higher viscosity than the solvent. The particles carry electric charges, which are, however, of relatively little importance to their stability (see below). Some carry positive and negative charges simultaneously, *e.g.*, a protein at its isoelectric point. The nature of the charge on a protein may be changed by altering the pH of the solution.

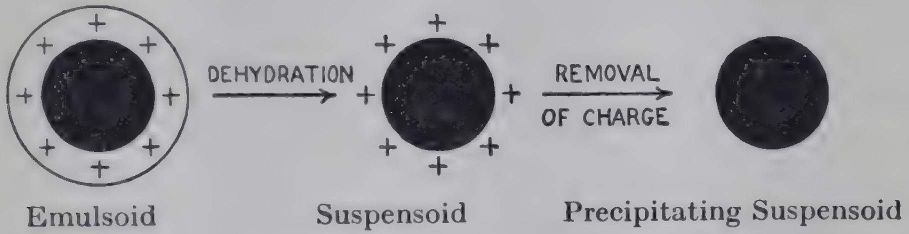


FIG. 7. "Salting out" of an emulsoid. (After Kruyt.)

Practically all the colloids of the living cell exist as emulsoids. Their most characteristic property is a great affinity for water. *Emulsoids readily adsorb water.* The molecules or particles become hydrated. This water is bound in the micelle and not free to move apart, and the emulsoid particles are probably either droplets of a concentrated solution or a molecule surrounded by a shell of adsorbed water (as in Fig. 7). It is this hydration which is mainly responsible for the stability of emulsoids. An emulsoid may be changed into a suspensoid by dehydration. The precipitation ("salting out") of emulsoid colloids such as proteins or starch by concentrated salt solutions is really an osmotic dehydration of the emulsoid, since the continuous phase is made strongly hypertonic and attracts water more strongly than the protein or starch molecule. By dehydration an emulsoid becomes a suspensoid with its characteristic instability, and is precipitated owing to removal of its charge by the salt present. This is represented in Fig. 7.

The process is reversible because of the affinity for water of the compounds which form emulsoids. How the particles regain their charge on re-solution is not known. Proteins can ionise, but molecules, like starch, cannot acquire a charge in this way.

The changes of state which an emulsoid can undergo are well illustrated by solutions of egg-white (mainly the emulsoid proteins albumin and globulin) in the following experiments :—

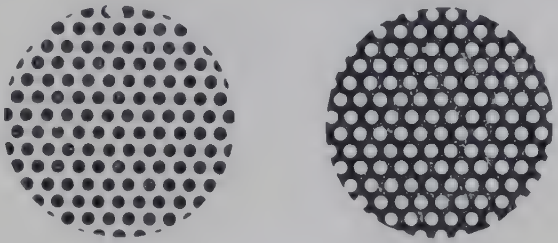
(1) Boiling a dilute solution when faintly alkaline produces no visible change, but the viscosity is diminished and surface tension raised. That means we have brought about the change : Emulsoid → Suspensoid.

(2) Boiling a similar dilute solution when very faintly acid causes precipitation, or coagulation. Here we have not only converted the emulsoid into suspensoid but precipitated the suspensoid, and we have done this by making the charge on the suspensoid neutral, for this precipitation only occurs near the isoelectric point of the proteins, *i.e.*, when they carry equal positive and negative charges. In alkaline or more acid solutions the protein ions are predominantly positively or negatively charged. Fig. 7, therefore, also represents this reaction, the dehydration in this instance being attained by heat.

(3) Heating undiluted egg-white to 100° C. causes it to set to a white semi-rigid jelly. This is a characteristic property of many emulsoids, and these two states are distinguished by the terms *sol* for the emulsoid solution and *gel* for the emulsoid jelly. With egg-white the changes are not reversible, because its proteins undergo an irreversible chemical change on heating. In many cases sol and gel formation is reversible.

Gel Formation. The change from a sol to a gel is a physical one, and is really an inversion of the two phases. This inversion can be brought about by changes of temperature, concentration of disperse phase, hydrogen ion concentration or salt concentration. In a sol the continuous phase is water (or a dilute solution) and the disperse phase the hydrated colloid (or concentrated solution). In a gel the concentrated solution forms the continuous phase and water (or dilute solution) the disperse phase. The dilute solution is entangled in a network of the concentrated solution. This is illustrated diagrammatically in Fig. 8.

Gel formation is probably most commonly observed in gelatin and agar-agar (p. 70). Solutions stronger than about 1% form gels on cooling. On warming, a sol is reformed and a gel on cooling again. Theoretically this change could be repeated any number of times. Actually in many cases, including those quoted, it cannot, because the colloid undergoes chemical decomposition on heating. As might be expected from the sluggish nature of the large particles, the changes are not sharp like the melting-point of a simple compound. A solution which gels at, say, 20° C., may not form a sol until warmed to 30° or 40° C. Most readers will have observed gel formation in strong solutions of soap and starch (starch or flour paste). The sol to gel change may be affected by a variety of conditions, such as pH or electrolyte content. Decrease of temperature does not always favour gel formation. Addition of $CaCl_2$ to an alkaline solution of caseinogen gives a sol which gels on heating. Egg-white forms a gel on heating.



Sol. Gel.

FIG. 8. Sol and gel. ○, water or dilute solution. ●, hydrated colloid.

Gels are quasi-solids with considerable resistance to deforming forces. They do not readily assume the shape of the vessel in which they are placed. It takes great pressure to squeeze water out of a gel, for the water has to push through the continuous phase of hydrated colloid. The form in which a table jelly comes from the grocer is an example of a typical gel. It contains about 33% of water and does not feel wet however hard it is squeezed; it also has considerable mechanical rigidity. To dry gelatin completely it is necessary to heat at 120° C. for a long time. Various reagents can be employed to make the continuous phase sufficiently "porous" for the water to be removed easily.

Formaldehyde solution is a familiar example. This is the main function of formaldehyde in the histological process of "fixing" tissues. It is probable that many tissue structures are essentially gels. A gel has many properties of the cell wall, *e.g.*, it permits the diffusion of simple molecules and offers little more resistance to diffusion than does water. The collodion membranes used for dialysis are, in fact, gels. The resistance of tissues to dehydration, especially the skin, strongly suggests the existence of gels. Some plants, *e.g.*, cacti, are abnormally resistant to dehydration ; this is largely due to their gel structure.

Imbibition. The resistance of gels to dehydration is reflected in their extraordinary affinity for water when they have been dried. Dried emulsoid colloids, particularly those which form gels readily, take up (imbibe) water and swell considerably ; this process is called *imbibition*. Considerable amounts of water may be taken up *without forming a liquid solution* (as calcium chloride crystals would). The first process in the germination of a seed is the taking up of water by imbibition. Imbibition can proceed against considerable resistance, as will be seen from the examples to be quoted. It has been calculated that during imbibition 1 g. of a dry gel could lift a 1-kg. weight 3·3 cm. Heat is liberated during the process. The avidity of these colloids for water is undoubtedly of importance in the passage of water into cells. Most dried animal and vegetable tissues show imbibition. The following examples illustrate the process :—

(1) Stephen Hales (1677–1759) placed some dried peas in an iron pot with a loose fitting cover, on which a 200-lb. weight was placed. When water was poured in through a small hole in the lid, it was imbibed with such force that the weight was lifted up.

(2) Before the days of explosives and proper quarrying tools, rocks were split by driving in dry wood wedges. Water was then poured on and the rock split by the force of imbibition.

(3) A stretched dry rope when wetted often imbibes the water so strongly that the rope breaks.

(4) The swelling of the wood of doors and windows in modern houses and the drawer that fits too well.

(5) One of the most rapid examples of imbibition is that of organic solvents by rubber. A strip of sheet rubber immersed in chloroform swells to several times its area in a few hours, and as quickly regains its original size on exposure to air.

(6) A therapeutic application of imbibition is the use of dried colloids as intestinal evacuants. By imbibing water and swelling they are

supposed to add bulk to the intestinal contents and so stimulate peristalsis, *e.g.*, agar-agar, p. 70.

This type of imbibition should not be confused with capillary imbibition as when water is absorbed by a sponge. This water is easily expelled by squeezing the sponge.

Protective Action of Emulsoids. Emulsoids can confer their stability to suspensoids. A small amount of gelatin added to a gold suspensoid renders the latter relatively resistant to precipitation by salts. This is known as the *protective action* of emulsoids. Presumably the emulsoid is adsorbed over the surface of the suspensoid, which becomes, in effect, an emulsoid and relatively stable. We have a somewhat analogous phenomenon in hydrotropy (below), where insoluble substances are made soluble by being surrounded with soluble molecules. These two factors probably account for many of the apparently abnormal solubilities of substances in the body. The protective action of colloids finds therapeutic application in the stabilisation of colloidal metals, *e.g.*, colloidal silver, which is a valuable antiseptic. Unprotected colloidal silver would be immediately precipitated by electrolytes in tissue fluids ; ionised silver, while an excellent antiseptic, is liable to cause pain.

HYDROTROPY (39)

It has frequently been observed that certain substances have the power of making water-insoluble * substances dissolve in water without any apparent chemical alteration of the dissolved substance. Moore and Parker, for example, in 1901 studied the solvent action of 5% aqueous solution of bile salts on the fatty acids (and their salts) from ox suet ; the solubility of mixed acids in water was less than 0·1%, in bile salt solution 0·5% ; the solubility was still further increased (to 4·0%) if 1% of lecithin was added, although lecithin itself is not truly soluble in water (but soluble in bile salts). The fatty acids could be readily recovered from solutions by extraction with solvents indicating that the fatty acid molecule had not been changed. In 1916 Neuberg suggested that substances having the power of making water-insoluble substances water-soluble should be called " *hydrotropic*

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* Insoluble is used here in the sense of very sparingly soluble.

substances." As examples of such substances, the cholic acids, benzoic acid, hippuric acid, phenylacetic acid and soaps of higher fatty acids may be quoted. The property is also shown by extracts of several organs. Among the insoluble substances which may be brought into solution are fats, phospholipides, sterols, calcium carbonate and phosphate, magnesium phosphate, strychnine and uric acid. The increase in solubility may be ten or a hundred times. The biological importance of the solution of insoluble substances by hydrotropic substances lies in the fact that *substances so dissolved are diffusible through membranes.* It is significant that in the body hydrotropic substances are found not only in bile but in intestinal juice, extracts of intestinal mucosa and in blood plasma, places where such substances would be particularly useful in assisting absorption and transport of insoluble substances such as cholesterol and fatty acids formed in digestion. Apart from the cholic acids in bile, the nature of these hydrotropic substances of the tissues is not known.

The hydrotropic action of a substance is not general. Bile salts are by no means universal dissolvers of insoluble substances. Further, there is no obvious relation between the chemical structures of the hydrotropic and the dissolved substances. In some cases the action might almost be described as specific. Filtration of the solutions through graded ultra-filters suggests that they contain both diffusible and non-diffusible complexes, the latter being present in the greatest amount in the most concentrated solutions; 100% diffusion is only attained in dilute solutions. As to the diffusible complexes, there is good evidence of a molecular relation between the hydrotropic and the dissolved substances. Thus when glycocholic acid and oleic acid diffuse together they do so in the molecular ratio 3 : 1. With desoxycholic acid crystalline complexes have been obtained with many substances, including fatty acids, benzene and camphor. In all these complexes there are several hydrotropic molecules to one of dissolved substance. One feature which appears to be common to all hydrotropic substances is that they have the power of decreasing surface tension. It would seem that the insoluble substances are dissolved by the hydrotropic substance owing to the surface force between them being greatly decreased. The formation of such complexes has been represented by Verzár and Kúthy, as in Fig. 9. This, even if not accurate, provides a convenient picture until a more exact structure is known.

This picture serves to emphasise the importance of a large amount of hydrotropic substance to dissolved substance for diffusion, for the smallest (*i.e.*, most readily diffusible) complex is the one with the highest percentage of hydrotropic substance. The picture has, however, the disadvantage that it conveys an impression of a physical union possible to any surface tension lowering substance; a hydrotropic substance will only dissolve *certain* insoluble substances.

The hydrotropic action of bile in digestion of fat is discussed on p. 244. An interesting example of hydrotropy is the solution of $CaCO_3$ in sodium salicylate. In an electric field part of the Ca goes to the positive electrode. (Ionised Ca goes to the negative electrode.) In blood part of the plasma Ca exists in colloidal form, which behaves like the $CaCO_3$—sodium salicylate hydrotrope in an electric field.

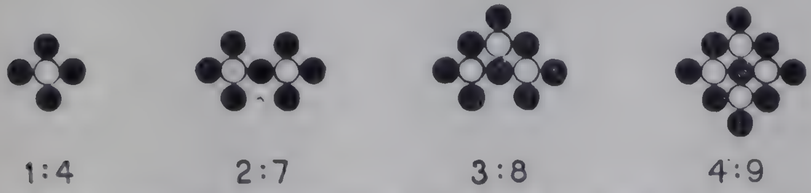


FIG. 9. Hydrotropes of fatty acid, ○, and bile acid, ●, of molecular ratios 1 : 4, 2 : 7, 3 : 8, 4 : 9. (After Verzár and McDougall, Ref. 39.)

CHAPTER V

CARBOHYDRATES (1, 4, 10, 18, 19, 20)

It is not easy to find a simple definition of the meaning of carbohydrate. The customary statement that a carbohydrate has an empirical formula which can be represented in terms of carbon and water $C_n(H_2O)_n$, or better, $C_m(H_2O)_n$, is not only inaccurate (*e.g.*, rhamnose, $C_6H_{12}O_5$) but misleading. Several other substances (*e.g.*, formaldehyde, acetic and lactic acids) have the same empirical formulæ. Nor does it make it any clearer to define m or n more specifically, since carbohydrates may have any number of carbon atoms from three upwards. The best working definition is, perhaps, the somewhat vague one that a carbohydrate is a simple sugar or a compound formed by combination of simple sugars. We shall see later that the term protein refers only to the complex formed by the combination of a large number of simple amino-acid units and not to the amino-acids ; the term carbohydrate covers not only the complex formed from many simple sugar units but also the simple sugar units themselves. It is most convenient to divide carbohydrates into three groups :—

(1) The simple sugar units or *Monosaccharides*.

(2) Compounds of two simple sugar units or *Disaccharides*. These compounds still have the properties of sugars.

(3) Compounds consisting of very many sugar units which no longer show the typical sugar properties—*Polysaccharides*.

There are, of course, intermediate tri- and tetra-saccharides, but these compounds we can omit, for we are concerned here only with those aspects of carbohydrate chemistry which are relevant to the study of *human* biochemistry. There are very many carbohydrates known to chemists ; only very few of these are utilised in the human body.

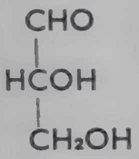
MONOSACCHARIDES

The simple sugars are colourless crystalline substances which have a sweet taste and elementary composition approximating to $(CH_2O)_n$. Chemically they can be regarded as polyhydric alcohols having reducing properties associated with a (potential) aldehyde or ketone group. The presence of secondary as well as primary alcohol groups gives rise to asymmetric carbon atoms, so that all sugars can exist in optically active form. The presence of an aldehyde or ketone group provides a subdivision into two types, known respectively as *aldoses* or *ketoses*. (The suffix -ose can nearly always be taken to indicate that a compound is a sugar or a carbohydrate.)

Since the properties of monosaccharides show marked differences according to the number of carbon atoms, we designate them *trioses*, *tetroses*, *pentoses*, *hexoses*, etc. Thus an aldopentose would contain four carbon atoms contained in alcohol groups and one in the aldehyde group.

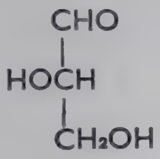
Chemistry of the Sugars

The simplest aldose conforming to our definition of a sugar is the triose glyceric aldehyde, or, in correct sugar terminology, glycerose.



$$\begin{array}{c} CHO \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

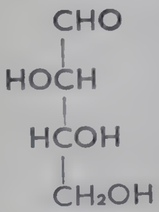
d-Glycerose



$$\begin{array}{c} CHO \\ | \\ HOCH \\ | \\ CH_2OH \end{array}$$

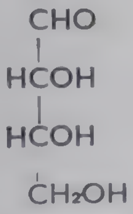
l-Glycerose

Since the secondary alcohol (= CH.OH) group is attached to an aldehyde group on one side and to a primary alcohol group on the other, its carbon atom is asymmetric, hence glycerose will exist in *d*- and *l*- forms, which can be represented as above. *d*- and *l*-Glycerose can be regarded as the parent substances of all the aldose sugars which are formed, theoretically, by insertion of further secondary alcohol groups. This will, of course, increase the number of isomers, thus *d*-glycerose will give two *d*-tetroses



$$\begin{array}{c} CHO \\ | \\ HOCH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

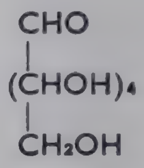
d-Threose



$$\begin{array}{c} CHO \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

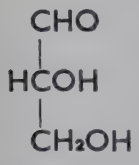
-Erythrose

whilst *l*-glycerose will give *l*-threose and *l*-erythrose. Similarly, by insertion of another secondary alcohol group *d*-threose and *d*-erythrose will each give two *d*-pentoses, making four in all. Each pentose gives two hexoses, so there are no less than eight possible hexoses formed from *d*-glycerose and eight from *l*-glycerose, sixteen in all. Since only three of these are pertinent to our study, we must refer the curious reader to the advanced text-books for the configurations of these sixteen sugars. Those of the three sugars will be given later. For the present it will suffice that the differences between the aldohexoses are due to the relative positions in space of the H and OH of the secondary alcohol groups, and that all sixteen aldohexoses can be represented :—



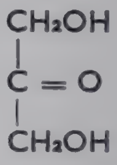
$$\begin{array}{c}\mathrm{CHO}\\|\\\mathrm{(CHOH)_4}\\|\\\mathrm{CH_2OH}\end{array}$$

Now let us refer to the ketose series. The triose corresponding to glycerose would be dihydroxyacetone,



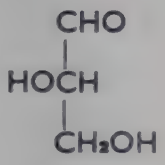
$$\begin{array}{l}\;\mathrm{CHO}\\\;|\\\mathrm{HCOH}\\\;|\\\;\mathrm{CH_2OH}\end{array}$$

d-Glycerose



$$\begin{array}{l}\mathrm{CH_2OH}\\|\\\mathrm{C=O}\\|\\\mathrm{CH_2OH}\end{array}$$

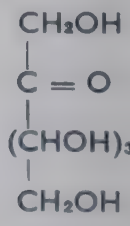
Dihydroxyacetone



$$\begin{array}{r}\mathrm{CHO}\;\\|\;\\\mathrm{HOCH}\\|\;\\\mathrm{CH_2OH}\end{array}$$

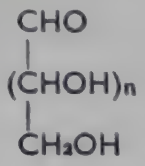
l-Glycerose

an isomer which can be formed from *d* or *l* glycerose (a general property of aldoses). Note that in the process an asymmetric carbon atom is lost, so that it is not till we get to the ketotetrose that optical activity appears. Consequently, instead of the sixteen isomers, as in the aldoses, we shall only have eight ketohexoses which can be represented by the general formula :—

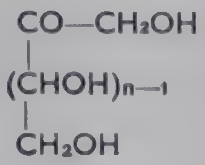


$$\begin{array}{c}\mathrm{CH_2OH}\\|\\\mathrm{C=O}\\|\\\mathrm{(CHOH)_3}\\|\\\mathrm{CH_2OH}\end{array}$$

The general formula for aldoses and the corresponding ketoses may be written :—



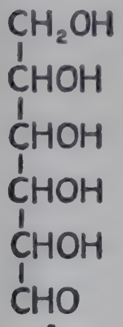
$$\begin{array}{l} CHO \\ | \\ (CHOH)_n \\ | \\ CH_2OH \end{array}$$



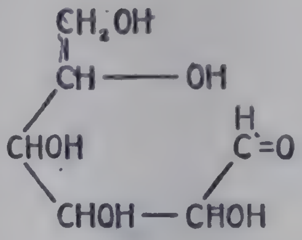
$$\begin{array}{l} CO—CH_2OH \\ | \\ (CHOH)_{n-1} \\ | \\ CH_2OH \end{array}$$

The Ring Structure of Sugars. Now we must examine these formulæ a little more closely. It is possible in the laboratory to treat an aldose (or ketose) so as to, in effect, insert a $=CH.OH$ group between the aldehyde group and its neighbouring $=CH.OH$. (Actually the aldehyde group is converted to $=CH.OH$ and a new aldehyde group formed.) Our formulæ above should then truly indicate the properties of the aldose, for there is no doubt that the formula given for glycerose is correct. Yet, in fact, pentoses and hexoses do not behave as would be expected. They are too stable and have not all the typical aldehyde properties, *e.g.*, they do not form an addition compound with sodium bisulphite or give Schiff's test. But most striking is their optical behaviour, for each of the isomers mentioned so far can exist in more than one form. Let us take *d*-glucose as an example. As soon as *d*-glucose is dissolved in water its specific rotation, $[\alpha]_D$, is $+ 111°$. On standing, especially in the presence of dilute ammonia, the value falls, finally remaining constant at $+ 52{\cdot}5°$, a phenomenon described as **mutarotation.** If, however, the *d*-glucose be first recrystallised from boiling pyridine, $[\alpha]_D$ on first dissolving in water is not $+ 111°$, but $+ 19°$; this solution also shows mutarotation, the specific rotation finally becoming constant at the same value, $+ 52{\cdot}5°$. In these two forms of *d*-glucose the only difference is due to stereoisomerism not revealed by our present formula. The two forms are referred to as α- and β-glucose respectively, and the solution giving $+ 52{\cdot}5°$ is an equilibrium mixture of the two forms. It must be clearly understood that these are stereoisomers of *d*-glucose and that β-*d*-glucose is not *l*-glucose, which has its own α and β forms, nor any of the other fifteen isomers mentioned. We have, therefore, to find a formula which gives us an additional asymmetric carbon.

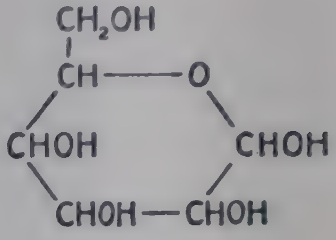
Let us for the moment restrict our inquiry to an aldohexose, and for the sake of simplicity omit the spatial representation of the H or OH round the known asymmetric carbons. So far we have been thinking of hexose as a straight chain of six carbon atoms as in **A** below.



A.



B.

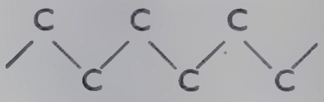


C.

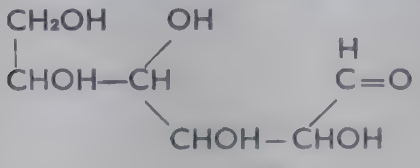
Aldehyde Form.

α or β Ring Form.

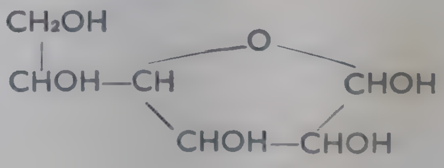
Now we must remember that the conception of a straight chain has risen solely because it is easier and quicker to draw on paper than the actual shape assumed by the carbon atoms in space. The carbon atoms could never assume a geometrically straight line, since they are connected by valencies at an angle equal to that formed by lines from the centre of a tetrahedron to two of its points. The nearest approach to a straight line would be this



and even such an arrangement would not normally exist, since the molecule would tend to occupy the smallest amount of space, *i.e.*, by forming a spiral. If now we redraw our formula, thinking, as we should always, of the molecule existing in space rather than on paper, we could legitimately claim **B** as a possibility. Obviously there is a strong tendency for such a molecule to assume a still greater stability by the simple process of closing the ring through an oxygen atom as in **C**. This arrangement gives a six-membered ring which chemists by experience know to be stable. We can also picture the formation of a five-membered ring in **D** and **E**, which would not be quite as stable. Other rings with seven, four, or even three members are theoretically possible.



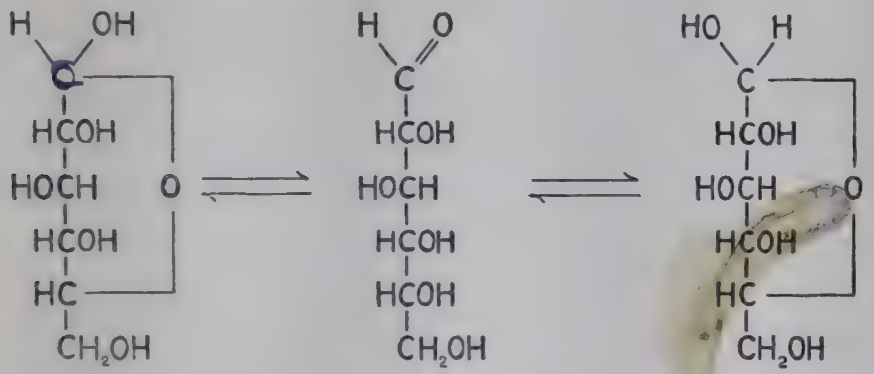
D.



E.

Now by forming these rings we have converted the aldehyde group into secondary alcohol, thus adding an asymmetric carbon atom (indicated by **C**) without upsetting the asymmetry of the original secondary alcohol group contributing to the oxygen link. We have thus lost an aldehyde group, but found the missing asymmetric carbon atom and explained the unexpected stability of hexoses.

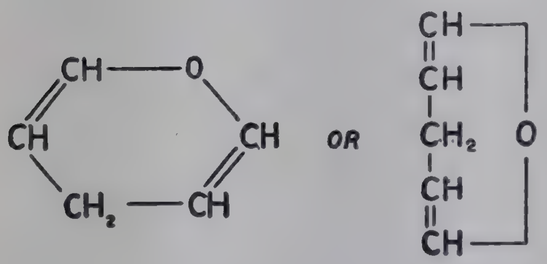
The aldehyde properties of a sugar are explained by the assumption that in solution there is always some of the aldehyde (or straight chain) form present, but so little that, at any given time, the characteristic reactivity of the aldehyde group is partly obscured. The mutarotation is explained as a reversible isomeric change, possibly involving the aldehyde form as an intermediate stage. To illustrate this we will write the formulæ for *d*-glucose in the straight chain form, in which the spatial arrangements of the H and OH are more easily appreciated. Ring formation is indicated by the long oxygen link.



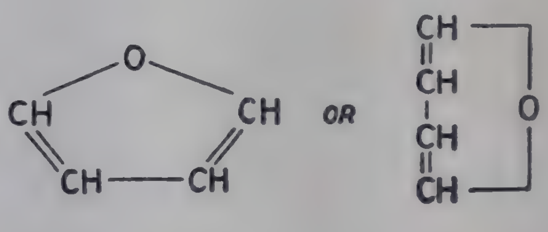
For some time there was doubt as to which ring form glucose assumed. Now we are sure that α- and β-glucose are represented by the six-membered ring form, or, as it is more frequently described, the *amylene oxide* form, since the oxygen joins a chain of five carbon atoms. We also know that the five-membered ring or *butylene oxide* form (as in **E** on p. 54) exists. This is sometimes called a γ-sugar, since the oxygen link is to the γ carbon atom as opposed to the δ carbon in the amylene oxide form. The greater reactivity of the γ- over the δ-form gave rise to the term "*active glucose*." Actually there is also evidence for the existence of propylene and ethylene oxide forms under certain conditions, some chemists even maintaining that all four forms in α- and β-modifications and the aldehyde form coexist in a solution of ordinary glucose. Since, however, the amylene oxide form greatly predominates we can for practical purposes regard ordinary glucose solution as an equilibrium mixture of α- and β-amylene oxide forms with sufficient aldehyde form to give certain aldehyde reactions ; it should not be considered a solution of a compound of fixed molecular structure.

The other sugars, both aldoses and ketoses, behave similarly.

Pyranose Furanose Nomenclature. The ring structure of sugars being established, it is more scientific to relate the nomenclature of sugars to the parent ring. Haworth has proposed a scheme in which all sugars forming six-membered rings are called *pyranoses* from their relation to pyrane, and those forming five-membered rings *furanoses* after furane.



PYRANE

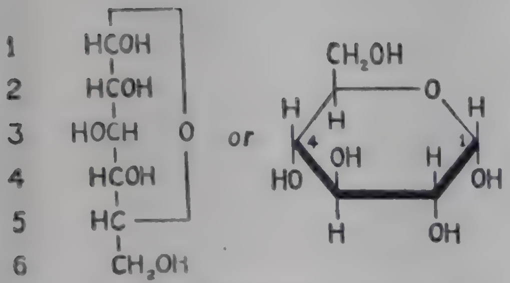


FURANE

Individual sugars are described by prefixing the main part of their common name, so that the amylene oxide form of glucose would be called glucopyranose and the γ- or butylene oxide form glucofuranose. The simplicity and lack of ambiguity of this nomenclature makes its use throughout this book desirable. Since, however, stereoisomeric differences are more readily perceived in the straight chain oxygen link formulæ, we shall adopt these rather than the more correct ring formulæ. If a sugar is referred to by its ordinary name only, the pyranose form is to be inferred. The pyranose and furanose forms of some sugars with their alternative names are set out on p. 57 in both types of formulæ.

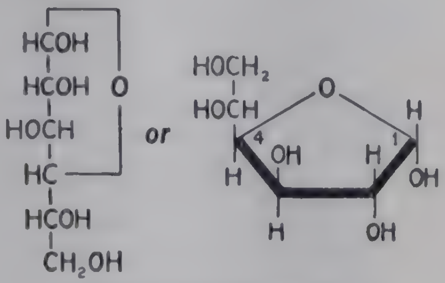
PYRANOSE SUGARS. FURANOSE SUGARS.

α-d-GLUCOSE.


1
2
3
4
5
6
HCOH
HCOH
HOCH
HCOH
HC
CH₂OH
O
or
CH₂OH
O
H
H
4
1
OH
H
HO
OH
H
OH

GLUCOPYRANOSE

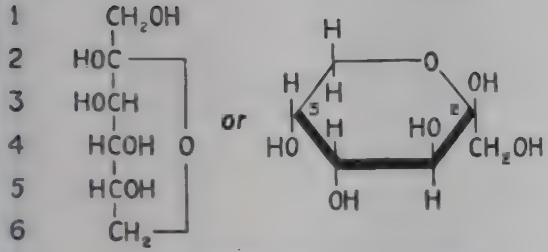
Ordinary Glucose
Amylene Oxide Form
δ-Glucose


HCOH
HCOH
HOCH
HC
HCOH
CH₂OH
O
or
HOCH₂
HOCH
O
H
4
1
OH
H
H
OH
H
OH

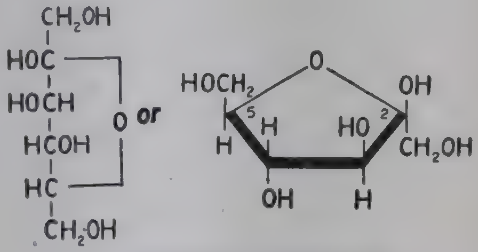
GLUCOFURANOSE

Active Glucose
Butylene Oxide Form
γ-Glucose

β-d-FRUCTOSE.


1
2
3
4
5
6
CH₂OH
HOC
HOCH
HCOH
HCOH
CH₂
O
or
H
O
OH
H
H
5
2
H
HO
HO
CH₂OH
OH
H

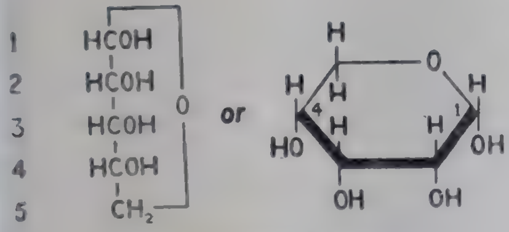
FRUCTOPYRANOSE


CH₂OH
HOC
HOCH
HCOH
HC
CH₂OH
O
or
HOCH₂
O
OH
5
H
HO
2
H
CH₂OH
OH
H

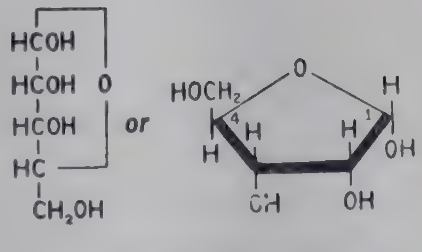
FRUCTOFURANOSE

γ-Fructose

α-d-RIBOSE.


1
2
3
4
5
HCOH
HCOH
HCOH
HCOH
CH₂
O
or
H
O
H
H
H
4
1
H
H
HO
OH
OH
OH

RIBOPYRANOSE


HCOH
HCOH
HCOH
HC
CH₂OH
O
or
HOCH₂
O
H
4
H
H
1
H
OH
OH
OH

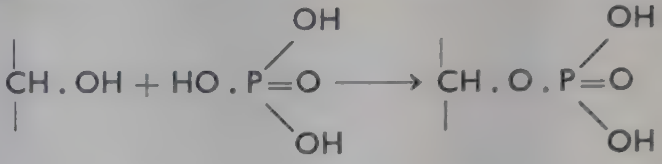
RIBOFURANOSE

γ-Ribose

General Properties of the Monosaccharides

A. Alcoholic. Sugars, by virtue of the alcohol groups, readily form esters with acids, all the free OH groups being replaceable.

The esters of greatest biochemical significance are those with phosphoric acid.

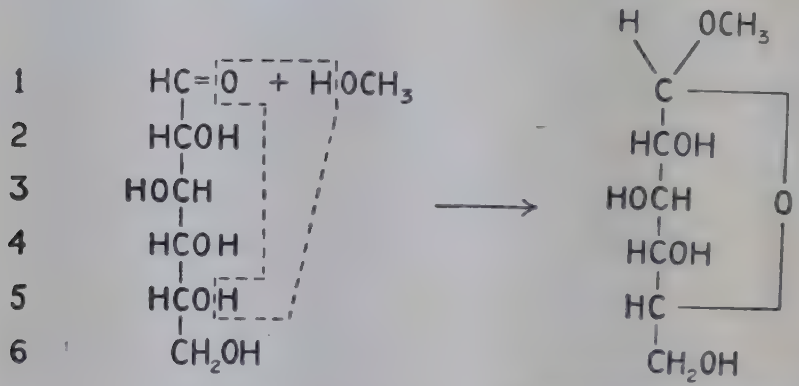


$$-\overset{|}{\underset{|}{\mathrm{CH}}}.\mathrm{OH} + \mathrm{HO}.\mathrm{P}(\mathrm{OH})_2{=}\mathrm{O} \longrightarrow -\overset{|}{\underset{|}{\mathrm{CH}}}.\mathrm{O}.\mathrm{P}(\mathrm{OH})_2{=}\mathrm{O}$$

We shall see later that hexose phosphates play an important part in the absorption and metabolism of carbohydrate. Pentose phosphates are involved in the make-up of nucleic acids.

B. Aldehydic or Ketonic. The reducing properties of the sugars are the result of the potential aldehyde or ketone group. The monosaccharides readily reduce alkaline copper, bismuth, or silver solutions, the copper to Cu_2O (*e.g.*, Fehling's test), the two others to the metallic state.

All reducing sugars will condense with dry methyl alcohol under the catalytic action of dry HCl to form **glycosides.*** Thus glucose forms α- and β-methyl glucosides.



α-METHYLGLUCOSIDE

The H is taken from carbon 5 because in space it is nearest to the aldehyde group, see B, p. 54.

Similarly, sugars form compounds with other OH containing substances such as alcohols, sugars, phenols, organic acids. Sugars are frequently found naturally in the form of glycosides; cerebrosides (p. 82) can be considered as glycosides, or more specifically galactosides. The disaccharides, we shall see shortly, are really glycosides. A complex glycoside frequently used therapeutically is *digitalin*. A glucoside which has been most

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* Glycoside is the general term for ethers formed by the reducing group of sugars. The ethers of specific sugars are called glucosides, galactosides, fructosides, etc.

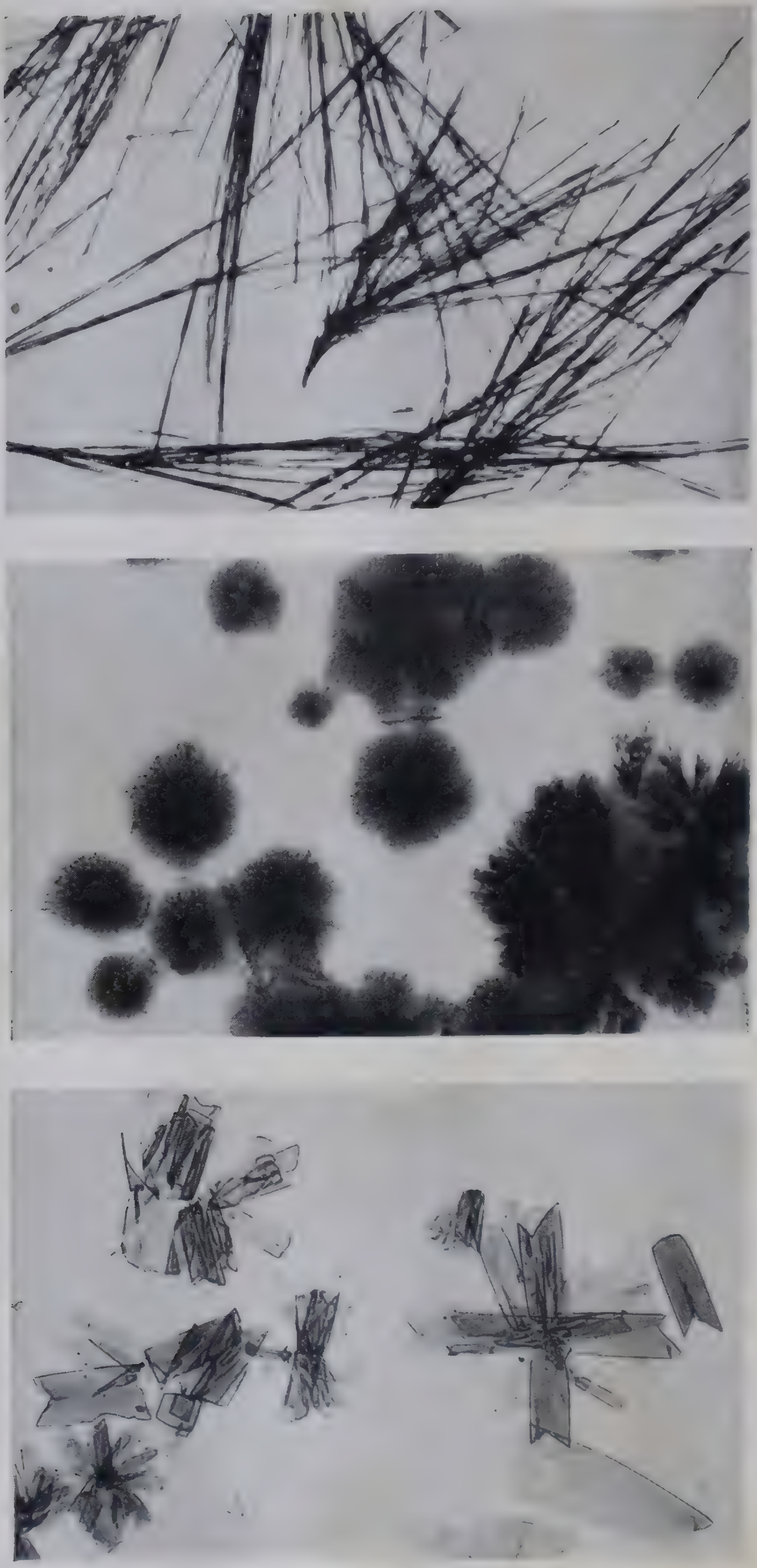
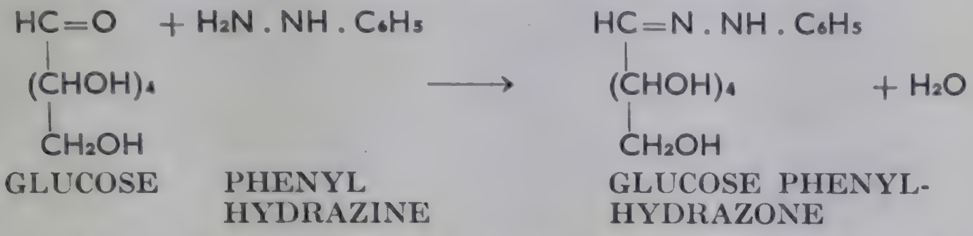


FIG. 10. Crystals of—
(*a*) Glucosazone. × 145.
(*b*) Lactosazone. × 145.
(*c*) Maltosazone. × 145.
(Not recrystallised.)

[*To face p.* 59.

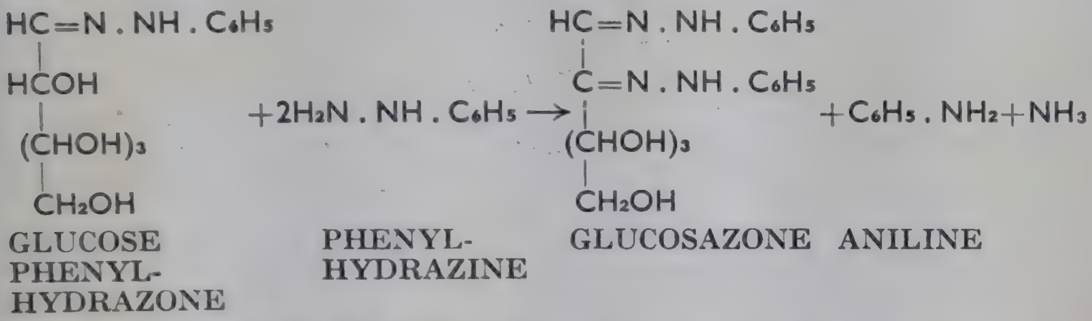
valuable in the experimental study of carbohydrate and protein metabolism is *phlorrhizin* (glucose and phloretin).

All reducing sugars condense with reagents such as hydroxylamine or phenylhydrazine. With phenylhydrazine in the cold a phenylhydrazone is formed.



$$\begin{array}{l} HC{=}O \\ | \\ (CHOH)_4 \\ | \\ CH_2OH \end{array} + H_2N\,.\,NH\,.\,C_6H_5 \longrightarrow \begin{array}{l} HC{=}N\,.\,NH\,.\,C_6H_5 \\ | \\ (CHOH)_4 \\ | \\ CH_2OH \end{array} + H_2O$$

At 100° C. in the presence of excess phenylhydrazine a further reaction takes place, involving carbon atom 2 in aldoses and carbon atom 1 in ketoses, which results in the formation of an osazone.

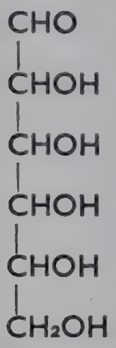


$$\begin{array}{l} HC{=}N\,.\,NH\,.\,C_6H_5 \\ | \\ HCOH \\ | \\ (CHOH)_3 \\ | \\ CH_2OH \end{array} + 2H_2N\,.\,NH\,.\,C_6H_5 \rightarrow \begin{array}{l} HC{=}N\,.\,NH\,.\,C_6H_5 \\ | \\ C{=}N\,.\,NH\,.\,C_6H_5 \\ | \\ (CHOH)_3 \\ | \\ CH_2OH \end{array} + C_6H_5\,.\,NH_2 + NH_3$$

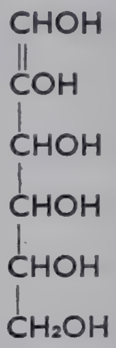
The sugar osazones are well-crystallised compounds, each with a characteristic form. Since the reaction involves the first two carbon atoms whose asymmetry is destroyed, fructose, glucose and mannose all form the same osazone. Galactosazone is quite distinct from glucosazone. The crystalline forms of sugar osazones are sufficiently characteristic to enable them to be used for identification (see Fig. 10), although such identification should be supported by a determination of the melting-point of a recrystallised specimen. More definite melting-points may be obtained with the compounds formed by 2 : 4-dinitrophenylhydrazine.

C. The Action of Alkali on Monosaccharides. The monosaccharides are very unstable in alkaline solution. By way of example we may confine our attention to glucose. In weak alkali α-glucose rapidly changes to a mixture of α- and β-glucose ; further, glucose undergoes intramolecular rearrangement, forming six other hexoses, including fructose and mannose (see p. 63).

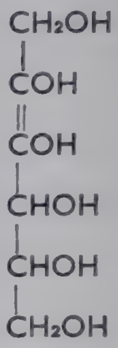
As the concentration of alkali increases, other products are formed, and it has been stated by Nef that glucose can form 116 different compounds in this way. The compounds of interest to us are those formed in the absence of oxygen, for the products are those found in the body as intermediates in carbohydrate metabolism. The reactions underlying these changes are probably the successive formation of unsaturated enol compounds, starting with the keto-enol change, the first compound formed being called glucose 1, 2-di-enol. By subsequent rearrangement the double bond could move, giving a 2, 3-di-enol and then a 3, 4-di-enol. All these di-enols are unstable and readily break at the double bond. In 0·5 N alkali (in the absence of oxygen) the 3, 4-di-enol is predominant. This breaks down in the first instance to glyceric aldehyde or dihydroxyacetone thus :—


$CHO-CHOH-CHOH-CHOH-CHOH-CH_2OH$

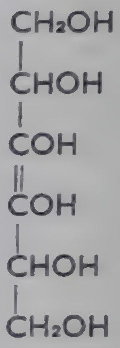
GLUCOSE


$CHOH=COH-CHOH-CHOH-CHOH-CH_2OH$

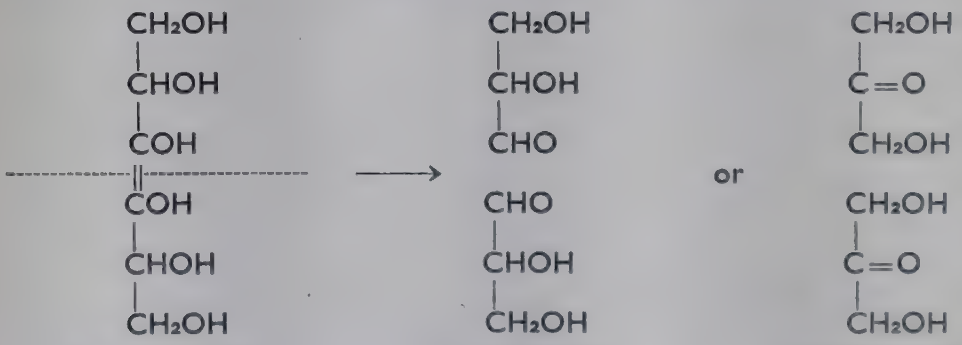
1, 2-DI-ENOL


$CH_2OH-COH=COH-CHOH-CHOH-CH_2OH$

2, 3-DI-ENOL


$CH_2OH-CHOH-COH=COH-CHOH-CH_2OH$

3, 4-DI-ENOL



3, 4-DI-ENOL

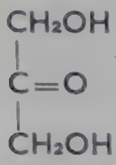
GLYCERIC ALDEHYDE

DIHYDROXY-ACETONE

We shall see later (p. 237) not only that the breakdown of glucose in muscle under anaerobic conditions involves the formation of these two trioses, but also that the reverse reaction takes place. In the presence of oxygen the breakdown of glucose in alkali is quite different.

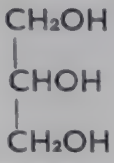
The Trioses and Related Compounds

In addition to the two trioses, several other three-carbon compounds are convertible to either glucose or lactic acid in the body. Since they are of importance as intermediates in metabolism, their formulæ are recorded here for convenience of reference :—



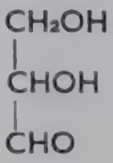
$$\begin{array}{l} CH_2OH \\ | \\ C=O \\ | \\ CH_2OH \end{array}$$

DIHYDROXY-ACETONE



$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

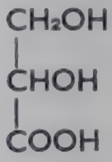
GLYCEROL



$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CHO \end{array}$$

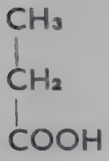
GLYCERIC ALDEHYDE

(Glycerose)



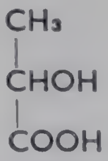
$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ COOH \end{array}$$

GLYCERIC ACID



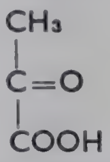
$$\begin{array}{l} CH_3 \\ | \\ CH_2 \\ | \\ COOH \end{array}$$

PROPIONIC ACID



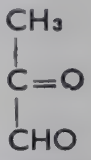
$$\begin{array}{l} CH_3 \\ | \\ CHOH \\ | \\ COOH \end{array}$$

LACTIC ACID



$$\begin{array}{l} CH_3 \\ | \\ C=O \\ | \\ COOH \end{array}$$

PYRUVIC ACID

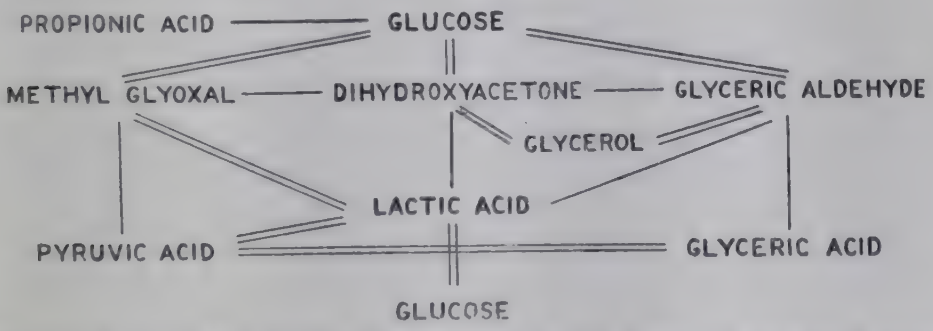


$$\begin{array}{l} CH_3 \\ | \\ C=O \\ | \\ CHO \end{array}$$

METHYL GLYOXAL

(Pyruvic Aldehyde)

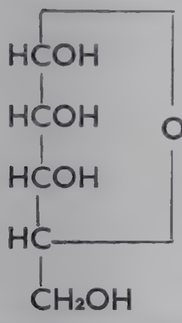
The biochemical interrelationships between these compounds will be seen later. For the moment we can summarise them diagrammatically in the scheme below, it being understood that the lines do not necessarily mean a direct reaction without the formation of intermediate products (*e.g.*, phosphorylated trioses). Many of these reactions are seen in the breakdown of muscle glycogen to lactic acid (p. 237). There is evidence that most of the reactions are reversible.



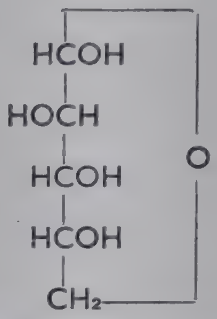
The reader may be interested to insert arrow heads on the lines when he finds evidence for the reactions elsewhere in this book.

Pentoses and Hexoses

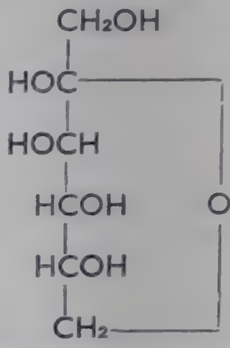
Apart from trioses, the only monosaccharides which we need consider are the pentose, ribose, and the hexoses—fructose, galactose, glucose and mannose. All are *d*-sugars,* only fructose is a ketose. Their formulæ are given in the following scheme :—



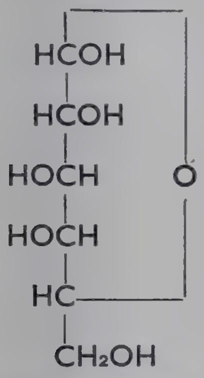
d-RIBOFURANOSE



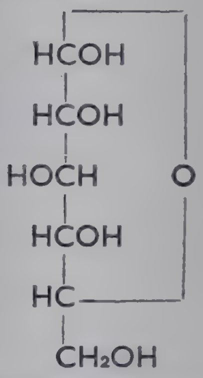
d-ARABOPYRANOSE



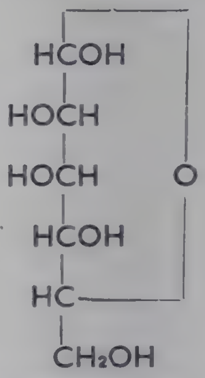
d-FRUCTOPYRANOSE



d-GALACTOPYRANOSE



d-GLUCOPYRANOSE

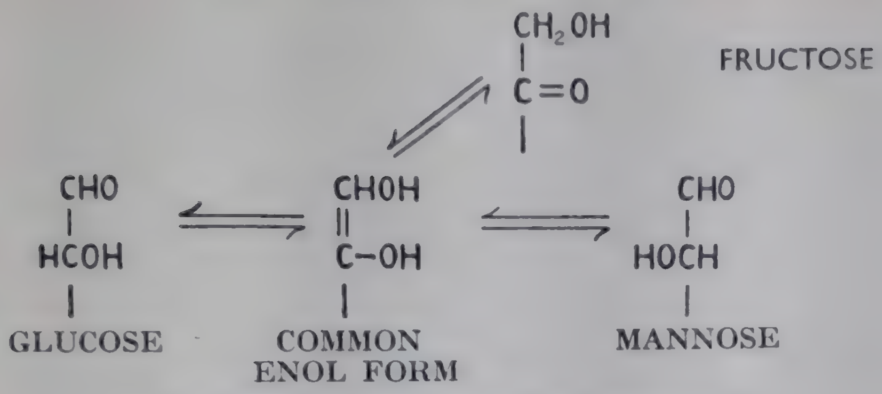


d-MANNOPYRANOSE

Note relationships between these sugars. Ribose and arabinose are the isomers formed by ascending from erythrose, and glucose and mannose are similarly formed from arabinose. Fructose is the ketose corresponding to glucose. Only galactose is derived from threose. The close relationship between fructose, glucose and mannose is emphasised by the fact that under the influence of dilute alkali each is converted into an equilibrium mixture of all three. It is believed that these three sugars form (from the aldehyde or ketone form) a 1, 2-di-enol compound which is common to all thus :—

---

* **Note.** *d*- and *l*- in modern nomenclature does not indicate that the compound is dextro- or lævo-rotatory, but the parent form from which the substance is derived, in this case *d*- or *l*-glycerose. Actually *d*-fructose is lævorotatory. This can be indicated thus : *d* (—) fructose. Glucose, which is dextrorotatory, would be written *d* (+) glucose.

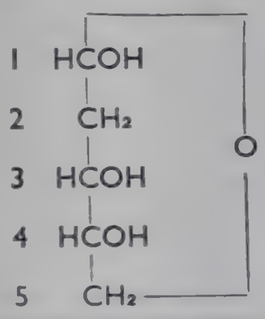

CH₂OH
C=O
FRUCTOSE
CHO
HCOH
GLUCOSE
CHOH
C–OH
COMMON ENOL FORM
CHO
HOCH
MANNOSE

(The four carbon atoms common to all are omitted)

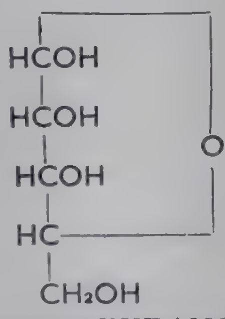
This may explain the ready utilisation of these three sugars by the body, for, once in the body, all are apparently rapidly converted into glucose, which can be regarded as the carbohydrate currency of the body. Galactose can also be utilised, but its conversion into glucose is not so clear. It can be synthesised in the body (*e.g.*, for galactolipides and lactose).

Pentoses

d-Ribofuranose is a constituent of adenylic, guanylic and inosinic acids (see p. 116) and of yeast nucleic acid. The sugar in thymonucleic acid is a derivative of *d*-ribopyranose, which has lost an oxygen atom from the second carbon atom, *i.e.*, it is a desoxypentose. Its correct name is **d-2-ribodesose,** since oxygen has been lost from carbon 2.


1 HCOH
2 CH₂
3 HCOH
4 HCOH
5 CH₂
O

d-DESOXYRIBOPYRANOSE


HCOH
HCOH
HCOH
HC
CH₂OH
O

d-RIBOFURANOSE

It has been stated that *d*-ribose is present in urine under certain conditions. *d-Arabinose* is rare in nature. The common form found combined in many gums is *l*-arabopyranose.

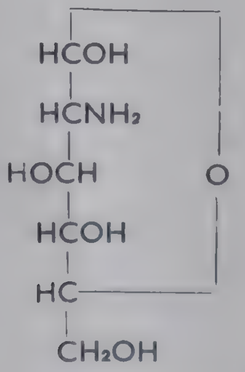
Hexoses

d-Fructose (*Lævulose, Fruit Sugar*). Free fructose is present in nearly all sweet fruits and honey. In combination, as a constituent of cane sugar or certain polysaccharides, it is widely distributed in the plant world. In the animal body it is apparently rapidly converted into glucose. It is most easily prepared by hydrolysis of its polysaccharide inulin. In distinction from other sugars fructose is soluble in hot absolute alcohol.

d-Galactose only occurs in combination. In the body it is a constituent of galactolipides, of certain proteins and of the sugar of milk, lactose. It is widely distributed in the form of polysaccharides in plants, particularly in seaweeds, lichens and mosses. It is usually prepared by hydrolysis of lactose.

d-Glucose (*Grape Sugar*, *Dextrose*) is, from our point of view, the most important of the sugars, since it is the form in which carbohydrate is transported in the blood. It is stored in the liver and muscles in polysaccharide form as glycogen. We shall see that the utilisation of glucose contributes very largely to the heat and energy output of the body by being burnt to CO_2 and H_2O. It is widely distributed in plant tissues and is present to the extent of about 0·1% in the blood and many tissues of animals. In combination with fructose as cane sugar it is found in many plants. In the polysaccharide starch it forms the chief carbohydrate store of plants, and as cellulose the chief supporting structure. It is prepared by hydrolysis of starch obtained from potato, maize, etc.

d-Glucosamine (2-*Aminoglucose*, *Chitosamine*). An interesting derivative of glucose, first found in chitin, the organic constituent of lobster shell, is glucosamine. It is apparently fairly widely distributed in nature, chiefly in combination with protein in glycoproteins. It closely resembles glucose in many properties, but has in addition weak basic properties, *i.e.*, it can form salts, *e.g.*, a hydrochloride.

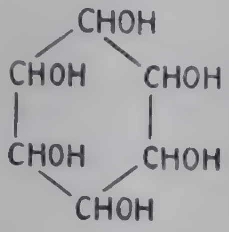


GLUCOSAMINE

2-Amino-d-galactose * (*Chondrosamine*) also found in glycoproteins has very similar properties.

d-Mannose does not occur free in nature, but is widely distributed in combination as the polysaccharide mannan, *e.g.*, in ivory nut. In the body it is found combined in certain proteins, *e.g.*, egg albumin.

Inositol (*Inosite*) may be mentioned here in that it was long known as "Muscle Sugar" and has empirical formula $(CH_2O)_6$. But it is not a monosaccharide, since it contains no aldehyde or ketone group and thus no reducing properties. Further, it has a ring of six carbon atoms.



INOSITOL

It has a very feeble sweet taste and occurs in muscle and other animal organs and in many plants. Its hexaphosphate is called *phytic acid* and CaMg phytate *phytin*.

URONIC ACIDS

If the primary alcohol group of a monosaccharide is oxidised to —COOH, a uronic acid is formed. This oxidation has not been accomplished in the laboratory directly; hexuronic acids have been synthesised indirectly with difficulty. The hexuronic acid from glucose, **glucuronic acid** (glycuronic acid), is apparently easily formed in the body, and is used to remove toxic substances such as camphor, phenols, etc., by forming conjugated glucuronic acids, which are readily excreted by the kidney (p. 316). Uronic acids are found in glycoproteins

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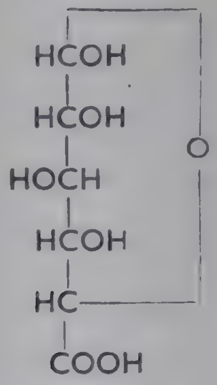
* The term galactosamine is usually applied to 6-aminogalactose, not 2-aminogalactose.

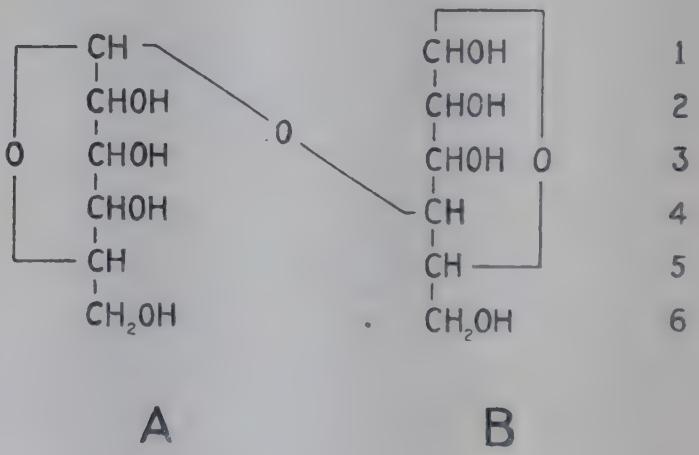
(glucuronic) and in certain polysaccharides (glucuronic. galacturonic, mannuronic).


HCOH
HCOH
HOCH
HCOH
HC
COOH
O

GLUCURONIC ACID

DISACCHARIDES

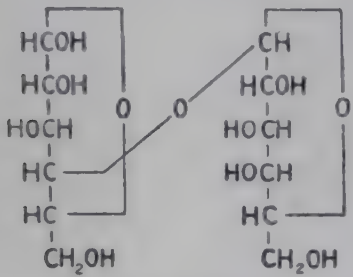
These can be regarded as products of the condensation of two monosaccharides with the elimination of H_2O, thus having a general formula $C_n(H_2O)_{n-1}$. This condensation involves attachment to at least one carbon atom in position 1, and consequently at least one potential aldehyde group will be lost. This can be


CH
CHOH
CHOH
CHOH
CH
CH₂OH
O
O
CHOH
CHOH
CHOH
CH
CH
CH₂OH
O
1
2
3
4
5
6
A
B

seen in the hexose **A** above. If the union with hexose **B** were with carbon atom 1, another potential aldehyde group would be lost and the disaccharide would be non-reducing. On the other hand, if the link is with carbon atom 4, as shown, **B** still retains its reducing properties and power of forming an osazone. Further, all reducing disaccharides, since they have carbon 1 in **B** free, will show mutarotation, for they can exist in α or β forms. The first carbon of **A** is fixed, so that we can have either the α or β form of **A**. In other words, the disaccharide above can exist in two distinct forms, each with α and β modifications—α (or β) **B**-α-**A** or α (or β) **B**-β-**A**.

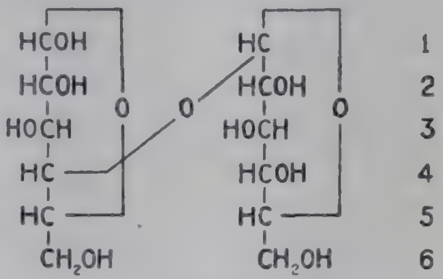
In general properties the reducing disaccharides are very similar to the monosaccharides except that the reducing power is not so strong, since there is now one reducing group to twelve carbons instead of six. All disaccharides are readily hydrolysed to monosaccharides by dilute acids. Of sixteen naturally occurring disaccharides only three are of physiological importance.

Lactose, composed of 1 mol. glucose + 1 mol. galactose.
Maltose, composed of 2 mols. glucose.
Sucrose, composed of 1 mol. glucose + 1 mol. fructose.



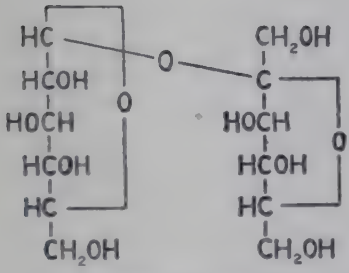
α-Glucose-β-Galactoside

α-LACTOSE



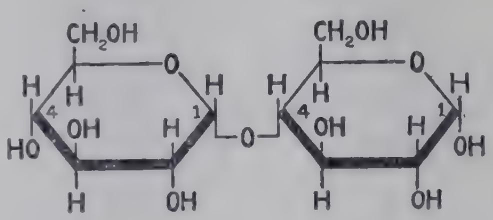
α-Glucose-α-Glucoside

α-MALTOSE



α-Glucose-?-Fructofuranoside *

SUCROSE



α-MALTOSE

From their formulæ it is easy to see why lactose and maltose have reducing properties, form different osazones and exhibit mutarotation. Sucrose, on the other hand, is a non-reducing disaccharide, forming no osazone and not showing mutarotation. Lest the reader have difficulty in visualising the spatial arrangement of disaccharides, the ring formula for maltose is also given. Note that in sucrose the fructose is in furanose form, although on hydrolysis fructopyranose is produced ; the labile fructofuranose changes to stable fructopyranose on hydrolysis.

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* It is not definitely known whether fructose is the α or β form.

Lactose (*Milk Sugar*) occurs in the milk of mammals. It must be formed by the animal, for any ingested lactose is hydrolysed before absorption, a special enzyme existing for this purpose.

Maltose (*Malt Sugar*) does not occur in the body, and only the products of its hydrolysis are absorbed. Its chief interest is as an intermediate product in the breakdown of starch in the alimentary canal. There is a special enzyme for the hydrolysis of maltose to glucose.

Sucrose (*Cane Sugar*) does not exist in the body, but is one of the sugars of our food. It is broken down by the enzyme invertase before absorption. During hydrolysis the specific rotation of sucrose (+ 66·5°) changes to − 19·5°, since the lævorotation of fructose is greater than the dextrorotation of glucose. The product of the hydrolysis used to be referred to as "*invert sugar*," hence the origin of the name of the enzyme.

POLYSACCHARIDES

The polysaccharides may be regarded as compounds consisting of a large number of monosaccharide units condensed together. The molecules are so large that true solutions are not possible, and consequently all give colloidal solutions with the exception of cellulose, which is insoluble. Attempts at determination of molecular weight have given most conflicting results. There is evidence that at least some polysaccharides are composed of relatively short chains of monosaccharide units (linked as in disaccharides) held together by co-ordination or co-valencies. The polysaccharides have neither sweet taste nor reducing properties.

Many types of polysaccharides are known ; they are described according to their constituent units, *e.g.*, *pentosans* $(C_5H_8O_4)_n$, *hexosans* $(C_6H_{10}O_5)_n$, or more specifically *glucosans*, *galactans*,* *mannans*,* *fructosans* indicating that the composing units are pentose, hexose, glucose, galactose, mannose or fructose respectively. Mixed polysaccharides are also known, some containing also derivatives of monosaccharides, such as aminohexoses (*e.g.*, glucosamine) and uronic acids.

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* Also called galactosans and mannosans.

The polysaccharides of physiological interest are *Cellulose*, *Glycogen* and *Starch*, which are all composed of glucopyranose units. Glycogen and starch, which are composed of α-glucopyranose units, are readily hydrolysed to glucose by boiling with dilute acids ; cellulose is only hydrolysed by strong acids and is formed of β-glucopyranose units.

Cellulose is a very stable insoluble compound, and, since it is the main constituent of the supporting tissues of plants, forms a considerable part of our vegetable food. It does not occur in the animal body. Herbivorous animals, with the help of bacteria, can utilise a considerable proportion of the cellulose ingested ; man cannot utilise any appreciable amount. Cellulose is, nevertheless, of considerable human dietetic value in that it adds " bulk " to the intestinal contents, thereby stimulating peristalsis and elimination of food residues. Filter paper is nearly pure cellulose.

Glycogen is the reserve carbohydrate of the animal, hence the description "Animal Starch." Any glucose stored in the body is synthesised into glycogen chiefly in the liver and muscles. Synthesis (glycogenesis) and hydrolysis (glycogenolysis) of glycogen occurs rapidly *in vivo*. *Postmortem* glycogenolysis is so rapid that no glycogen, but only glucose, can be detected in the liver or in muscle two or three hours after death. Glycogen is found in plants which have no chlorophyll system, *e.g.*, fungi and yeasts, but not in green plants. There is probably more than one glycogen ; a rabbit fed with glucose forms a glycogen composed of chains of twelve glucopyranose units, whereas from galactose a glycogen with chains of eighteen units is formed. The latter is not so easily hydrolysed *in vivo*. Curiously mixtures of the twelve and eighteen glycogens have not been encountered.

Glycogen is readily soluble ; its solutions are usually opalescent and give a red-brown colour with iodine.

Starch plays in chlorophyll-containing plants the same *rôle* as glycogen does in animals—that of an easily controlled store of carbohydrate. In plants the starch is laid down in the cells in granules with concentric stratifications which are characteristic of the species of plant and form a means of detecting the origin of a starch.

Starch is more complex than glycogen and is a mixture of two substances of similar structure—*amylose* and *amylopectin*—which are both composed of chains of twenty-four to thirty glucopyranose units and probably differ mainly in the degree of aggregation. Their molecular weights (by ultracentrifuge) are about 60,000 and 300,000 respectively.

Raw starch is insoluble in cold water, due to the resistance of the outer cellulose layer of the granule. When this is ruptured, *e.g.*, by heating in water, starch is soluble. Very finely ground starch may be partly soluble in cold water, the layer having been ruptured during grinding. Even raw starch is readily soluble in boiling water. Concentrated solutions gelatinise on cooling and are used as an adhesive—starch paste. Dilute solutions of starch are opalescent. The so-called *soluble starch*, which gives a clear solution, is a partially hydrolysed product and does not gelatinise on cooling. Both give an indigo blue colour with iodine.

Dextrins. Starch is quickly hydrolysed to glucose by boiling with dilute acid. When hydrolysed by an enzyme, *e.g.*, malt diastase or ptyalin, intermediate products can be detected. The first products are ill-defined substances called dextrins, with varying behaviour towards iodine. *Amylodextrin*, *erythrodextrin* and *achroodextrin* give blue, red-brown and no colour respectively, achroodextrin being the simplest. If they have reducing properties at all, they are very feeble. They have, however, a faint sweet taste. As they form sticky solutions in water, they are frequently used as adhesives, *e.g.*, on postage stamps.

The final product of hydrolysis of starch by ptyalin or diastase is maltose, which is only hydrolysed (enzymically) to glucose by a special enzyme maltase. (See pp. 216–217.)

Amongst the other polysaccharides are :—

Agar-agar, a galactan found in certain seaweeds and employed as a culture medium in bacteriology. It is frequently used therapeutically (p. 47).

Gums are mainly pentosans associated with hexosans.

Inulin, found in tubers of the Jerusalem artichoke, is a fructosan with fructofuranose in chains of about 30 units.

Pectins, the substances found in most fruits and supposed to be responsible for the "setting" of jam, are polygalacturonic acids. Some galactose units may also be present. The arabinose found after hydrolysis is derived from impurities.

Of these, inulin resembles starch, but the others are more like cellulose in that they are not easily hydrolysed by amylases, although readily hydrolysed by dilute acids ; hence they are called *hemicelluloses*.

CHAPTER VI

FATS AND RELATED COMPOUNDS (1, 4, 10, 21, 22, 23, 24)

Chemically, fats are the fatty acid esters of the trihydric alcohol glycerol. The term fat, however, has been so widely used to indicate substances of a fat-like nature that if we want to speak of glycerol esters of fatty acids we must say "*neutral* (*or true*) *fat*." The confusion has largely arisen through practical difficulties. When a tissue or food is analysed the first stage is to separate fat from carbohydrate and protein by extracting with a "fat-solvent" such as ether. Evaporation of the extract leaves a greasy mass which is often extremely complex and can only be separated into its many constituents by the expenditure of much time and expert labour. Consequently it has been the custom when analysing food to weigh the greasy mass and record that as the amount of fat present.

Attempts at classification of fat-like substances involving the terms lipoid and lipin have been so confused by misuse as to make their adoption unsatisfactory. The lipide classification given below is more satisfactory and embraces all substances which are extractable from a tissue by fat solvents and which are in any way chemically related to true fats.

Classification of Lipides

(After Bloor, *Chem. Rev.*, 1925, 2, 243)

Lipides are substances with the following characteristics :—

(*a*) They are insoluble in water but soluble in fat solvents such as ether, chloroform and benzene.

(*b*) They are ester-like compounds of fatty acids or capable of forming such esters.

(*c*) They are utilised by living organisms.

I. Simple Lipides. (Esters of fatty acids with alcohols.)

(1) *Fats.* (Esters of fatty acids with glycerol.)

(2) *Waxes.* (Esters of fatty acids with alcohols other than glycerol.)

II. Compound Lipides. (Esters of fatty acids with alcohols and other groups.)

(1) *Phospholipides* or *Phosphatides.* (Substituted fats containing phosphoric acid and nitrogen.)
(2) *Glycolipides* or *Cerebrosides.* (Compounds of fatty acids with carbohydrate and containing nitrogen but not phosphoric acid.)
(3) *Aminolipides*, *Sulpholipides*, etc. (Not sufficiently characterised for classification.)

III. Derived Lipides. (Substances derived from the above groups by hydrolysis.)

(1) *Fatty Acids* of various series.
(2) *Sterols* and other alcohols of high molecular weight which are found in nature in combination with fatty acids.

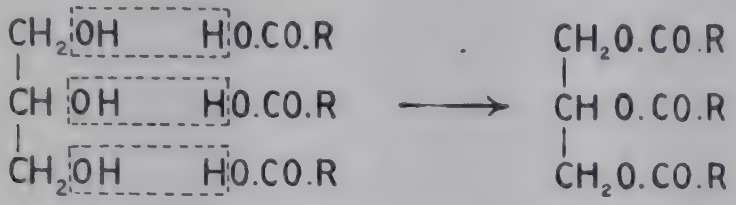
SIMPLE LIPIDES (1, 4, 21)

1. True Fats

A fat is liquid or solid at ordinary temperatures according to its melting-point. The substance we call olive oil is strictly olive fat. Coco-nut oil is also a true fat, and is a solid at the temperatures predominating in this country; it owes its name to the liquid form in which it exists at its place of origin in the tropics. Oil is a confusing term, since it also connotes substances having no relation to fats, such as the mineral lubricating oils, which are hydrocarbons. (Some lubricating oils, however, are actually fats, *e.g.*, castor oil.) Further, many hydrocarbon oils on cooling form (physically) fat-like solids, *e.g.*, vaseline.

The term oil must therefore be taken as indicating only the physical state of the substance, and must never be regarded as defining a chemical group.

True fats are esters of the alcohol glycerol with various fatty acids of general formula R.COOH, where R represents the fatty acid molecule less its —COOH. Since all three hydroxyl groups of glycerol are esterified, fats are triglycerides ; if R.COOH be butyric acid the fat is called tributyrin and so on.



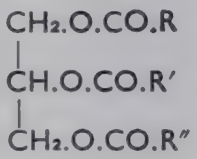
$$\begin{array}{l} CH_2\,\boxed{OH \quad H}\,O.CO.R \\ | \\ CH\,\boxed{OH \quad H}\,O.CO.R \\ | \\ CH_2\,\boxed{OH \quad H}\,O.CO.R \end{array} \longrightarrow \begin{array}{l} CH_2O.CO.R \\ | \\ CH\,O.CO.R \\ | \\ CH_2O.CO.R \end{array}$$

GLYCEROL. FATTY ACID. TRIGLYCERIDE.

The commonest fatty acids in natural fats like butter, lard, suet, tallow, olive oil, are :—

Palmitic acid . .	$CH_3.(CH_2)_{14}.COOH$
Stearic acid . .	$CH_3.(CH_2)_{16}.COOH$
Oleic acid . . .	$CH_3.(CH_2)_7.CH{=}CH.(CH_2)_7.COOH.$

Other fatty acids are usually present in smaller amounts. Until recently it was thought that natural fats consisted chiefly of mixtures of the triglycerides, tripalmitin, tristearin and triolein, since pure triglycerides are never found in nature. However, the failure of several investigators to isolate appreciable quantities of these triglycerides from fats has led to the conclusion that natural fats are really mixtures of mixed triglycerides represented by the general formula :—



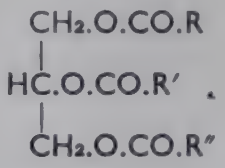
$$\begin{array}{l} CH_2.O.CO.R \\ | \\ CH.O.CO.R' \\ | \\ CH_2.O.CO.R'' \end{array}$$

in which R, R′ and R″ represent three different fatty acid residues. Many such mixed triglycerides have been isolated, *e.g.*, oleopalmitobutyrin from butter, stearodiolein from human fat, palmitodistearin from lard and beef tallow.

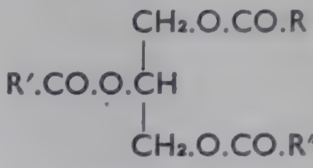
Note that a mixed triglyceride presents not only possibilities of isomerism due to the relative positions of the various acids, which can be represented thus

—R —R′ —R″	—R —R″ —R′	—R′ —R —R″

but also will have an asymmetric carbon atom and form optical isomers unless the outside acids are the same, *e.g.*,



$$\begin{array}{l} \mathrm{CH_2.O.CO.R} \\ \;\;| \\ \mathrm{HC.O.CO.R'} \\ \;\;| \\ \mathrm{CH_2.O.CO.R''} \end{array}$$



$$\begin{array}{r} \mathrm{CH_2.O.CO.R} \\ | \;\;\;\;\;\;\;\;\;\;\;\;\;\; \\ \mathrm{R'.CO.O.CH} \\ | \;\;\;\;\;\;\;\;\;\;\;\;\;\; \\ \mathrm{CH_2.O.CO.R''} \end{array}$$

Curiously the only optically active compounds of this type found naturally are those containing optically active fatty acids (*e.g.*, castor oil and chaulmoogra oil). Mono- and di-glycerides have been prepared synthetically but do not occur in nature.

The fatty acids which have been isolated from natural glycerides include all the saturated acids on p. 83 from butyric to lignoceric, and all the unsaturated acids except nervonic and ricinoleic acids.

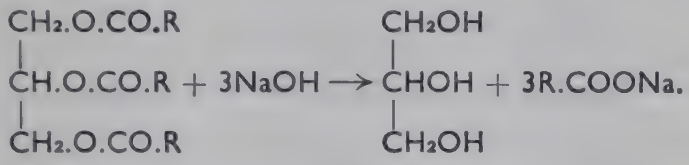
Physical Properties of True Fats. The true fats are insoluble in water, but readily soluble in " fat-solvents," *e.g.*, ether, chloroform, benzene, petroleum-ether, and carbon tetrachloride. They are readily soluble in hot alcohol, but only slightly so in cold. The fats themselves are good solvents for other fats, fatty acids, etc. They should be tasteless, odourless, colourless and neutral in reaction, but after exposure to air for a sufficient length of time they usually become acid and yellow owing to partial hydrolysis and oxidation of unsaturated fatty acids, and are said to be rancid (see p. 76). Several neutral fats can be readily crystallised *e.g.*, beef, mutton and pork fat.

Their *melting points* are low, but always higher than the temperatures at which they solidify again (*setting points*). Thus beef fat melts at 49·5° C., but sets again at 36°. This is not due to the mixed nature of the fat, for pure tristearin melts at 71·5° and sets at 52·5°. The glycerides of unsaturated fatty acids have lower melting-points than those of the corresponding saturated acids. Human fat melts about 17° C. The specific gravity of solid fats is about 0·86 ; and of the liquid fats 0·915–0·94. One consequence of the low specific gravity of fats is that fat people float in water more readily than thin ones.

Spreading. A liquid fat placed on water spreads uniformly over the surface of the water, and if the quantity is sufficiently small will form a layer 1 molecule thick. One effect of this spreading is to lower surface tension. This is of importance in the vital mechanics of protoplasm.

Chemical Properties

(1) **Hydrolysis.** Whilst most fats undergo slight partial hydrolysis spontaneously, they are only completely separated into their constituents by heating with superheated steam, or boiling with acids or alkalis. Fats are not appreciably hydrolysed by boiling with water alone, a fact applied commercially and domestically in the separation (rendering) of fat from meat trimmings, *e.g.*, in the preparation of lard. Hydrolysis by boiling with alkali is termed **saponification,** since the alkali salts or soaps of the fatty acids are formed.



$$\begin{array}{l} CH_2.O.CO.R \\ | \\ CH.O.CO.R \\ | \\ CH_2.O.CO.R \end{array} + 3NaOH \rightarrow \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + 3R.COONa.$$

FAT. GLYCEROL. SOAP.

Since the common fats contain predominantly palmitic, stearic and oleic acids, the soap we use for washing consists largely of the sodium salts of these acids. Whilst these fatty acids are insoluble in water, their sodium and potassium salts are soluble. In dilute solution soaps undergo partial hydrolysis :—

$$R.COONa + H_2O \rightarrow R.COOH + NaOH$$

This explains why the clear strong solution of a pure soap, *e.g.*, Na stearate, becomes cloudy on dilution, for the higher fatty acids are insoluble. It also accounts for the alkalinity of ordinary soap solutions. The NaOH formed from soaps in this way has a deleterious effect on the skin. This disadvantage is obviated in toilet soaps, either by adding excess of fatty acids, or employing a large proportion of Na oleate which is not so readily hydrolysed by water.

Calcium and magnesium soaps are insoluble. The diminished efficiency of soaps in hard water is due to the precipitation of the fatty acids by the calcium salts of the water. The solubility of sodium and potassium soaps is considerably diminished by the presence of excess Na or K ions, hence the difficulty of washing with ordinary soap in sea-water. A sodium soap is completely insoluble in saturated NaCl ; soaps are separated ("*salted out*") in this way after saponification of a fat.

(2) **Addition Reactions.** Fats containing, as all natural fats do, some proportion of unsaturated fatty acid, behave as unsaturated

substances, and readily add on other elements such as halogens. Under suitable conditions a fat can be made to take up iodine quantitatively at each double bond thus

$$-\mathrm{CH}=\mathrm{CH}- \rightarrow -\mathrm{CHI.CHI}-$$

The number of grams of iodine so taken up by 100 g. of a given fat is called its "**iodine value**" and affords a simple way of assessing the degree of unsaturation and, therefore, the reactivity of the fat. The iodine values of some common fats are given in the table on p. 77.

Under the influence of a suitable catalyst, such as finely divided nickel at a high temperature, unsaturated fats can combine with hydrogen and become more saturated. For example, addition of 2H to oleic acid would give stearic acid. In this way edible fats of high dietetic value can be made from cheap and comparatively inedible oils, such as cottonseed or fish oils. The hydrogenation is not, of course, carried to completion, as this would yield a high melting, hard, unpalatable fat. The best known product of this kind, *margarine*, has an iodine value slightly higher than butter and still contains a high proportion of oleic acid. Many lard substitutes are made by this method.

(3) Oxidation. Unsaturated fatty acids react with oxygen to form a number of substances including aldehydes and ketones, which may react further to form complex resinous products. These changes occur fairly rapidly on exposure to air if the fat be sufficiently unsaturated. Linseed or tung oils, when spread in thin layers on a surface, are rapidly converted to tough elastic waterproof films which adhere closely. For this reason such oils are known as *drying oils*. Their principal use is in the manufacture of paints, varnishes, linoleum, etc.

Rancidity. This oxidation process occurs to a greater or lesser extent in nearly all natural fats exposed to air, light and moisture, particularly if warm, *e.g.*, in summer. In addition there is partial hydrolysis causing liberation of fatty acid, which apparently accelerates the oxidation processes. The rancidity of stale fats can be ascribed to these changes, the peculiar taste and odour being due in some cases to the liberation of free volatile fatty acid, *e.g.*, butyric acid from butter, and in others to the production of odoriferous aldehydes or ketones by oxidation. This spontaneous oxidation can be considerably inhibited by the presence of traces of several organic compounds, especially certain phenols. Such substances are termed *anti-oxidants* and are present in many vegetable oils. This explains the tendency of certain oils to keep better than others with the same iodine value.

Biological Importance. True fats form reserve foods both in animals and plants, and are the most compact form in which energy can be stored, the oxidation of 1 g. of fat providing more than twice the energy of 1 g. of carbohydrate. Fats of varying degree of saturation are found in different parts of the body. The fat depots provide the most saturated.

The bulk of true fat in the body is located in subcutaneous and retroperitoneal tissues. The composition, *e.g.*, degree of saturation, of this store fat varies in different species and may even be modified by environment or diet. This fat in pigs and rabbits in captivity is more saturated than that in the wild animals. The iodine value of the fat of the domesticated rabbit is 64, whereas the value for the wild animal is 101.

Analysis of Fat. The reader will probably have inferred from the foregoing that naturally occurring fats are so complex that it is impracticable to specify the presence of any particular triglyceride. Instead, we have to define our fat by the properties of the mixed fatty acids. Of the six analyses usually made by the professional analyst we need only consider two, the **iodine value** already referred to, and the **saponification value**, or number of milligrams of KOH used up in the saponification of 1 g. of fat. The saponification value gives, in effect, the mean molecular weight of the fatty acids. A high saponification value indicates a greater proportion of fatty acids of low molecular weight, since for a given weight of acid the simpler fatty acids take up more KOH than the higher fatty acids. Typical values are given in the table.

Fat	Iodine Value	Saponification Value
Coco-nut oil	9	246–260
Butter	25–50	220–233
Beef fat (tallow) . . .	36–48	192–200
Pork fat (lard)	50–70	195–197
Human fat	57–66	193–199
Olive oil	78–90	185–195
Cottonseed oil	106–112	192–196
Cod-liver oil	144–168	175–193
Linseed oil	192–195	190–195

2. Waxes

Esters of fatty acids with higher alcohols which usually only have one hydroxyl group are described as waxes. They are formed as secretions, which are mostly protective in function, by many animals. Like the true fats the natural waxes are complex mixtures. In properties they resemble the fats, although they are not so readily hydrolysed, and are usually solid. Three common waxes and their chief constituents are :—

Bee's wax	Myricyl ($C_{30}H_{61}OH$) palmitate.
Lanoline	Cholesterol (p. 86) palmitate, stearate and oleate.
Spermaceti (found in the skull of the sperm whale).	Cetyl ($C_{16}H_{33}OH$) palmitate.

In the human body the commonest waxes are esters of cholesterol ; they are most abundant in blood plasma (83–164 mg. per 100 c.c.), the suprarenal glands and sebaceous glands.

COMPOUND LIPIDES (1, 4, 10, 22)

(1) Phosphatides (Phospholipides)

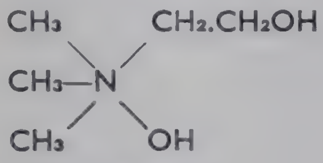
Phosphatides, while especially abundant in the brain, are present in every animal and plant cell and considerable evidence has accumulated to show that they play an essential and very important part there. The layer bounding the surface of the cell is probably composed essentially of phosphatides, which, by their unique properties, influence the transference of substances through the cell membrane ; the formation of phosphatides is usually admitted to be an intermediate stage in the utilisation of fat ; the marked conservation of phosphatides during extreme starvation is evidence of their essential nature, for death supervenes before appreciable amounts of phosphatides are lost, whereas carbohydrates, simple lipides and even proteins may be very considerably diminished.

The phosphatides, whilst having many properties in common with the simple lipides, are characterised by containing both phosphoric acid and a basic nitrogenous group. Their sparing solubility in acetone serves for their separation from simple and derived lipides which are readily soluble in acetone.

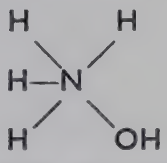
Like true fats, the phosphatides are really complex mixtures of very similar compounds. So far only three types have been identified with certainty—*lecithins*, *kephalins* and *sphingomyelins*.

Lecithins. Freshly prepared lecithin is a pale yellow waxy solid which has not been obtained crystalline. On exposure to air and light, oxygen is rapidly absorbed with a darkening of colour. Water is also readily absorbed and the mass becomes plastic, so that an old specimen of lecithin appears a black greasy mass. Fresh lecithin in contact with water swells up, and if sufficient water is present forms a slimy emulsion or colloidal solution, a property which suggests a possible mechanism for the transport of fatty substances in aqueous body fluids. If the process is observed under the microscope it is seen that the mass of lecithin appears to bud and put out complicated outgrowths called "*myelin*" forms. This phenomenon is attributed to a tendency to bring more lecithin molecules into contact with water. Like the fats, lecithin shows the property of spreading to a monomolecular film on water.

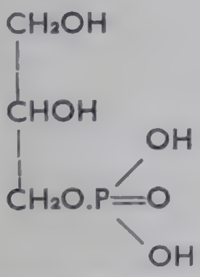
Phosphatides are readily hydrolysed by boiling with acid or alkali. Hydrolysis of a lecithin with NaOH yields glycerol, soaps, sodium phosphate and an organic base called **choline,** which is a substituted ammonium hydroxide having both basic and alcoholic properties. Careful partial hydrolysis of lecithin results in the formation of intermediate products, from which it can be concluded that the four constituents are linked in the order, fatty acids—glycerol—phosphoric acid—choline. The glycerol is combined with phosphoric acid as glycerophosphoric acid, which can exist in two forms.



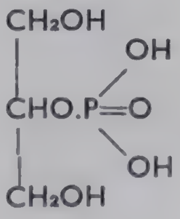
CHOLINE



AMMONIUM HYDROXIDE

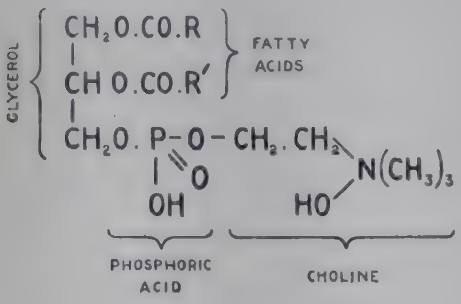


α-GLYCEROPHOSPHORIC ACID

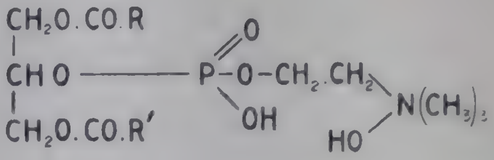


β-GLYCEROPHOSPHORIC ACID

Actually lecithins corresponding to both forms exist and can be represented :—



α-LECITHIN



β-LECITHIN

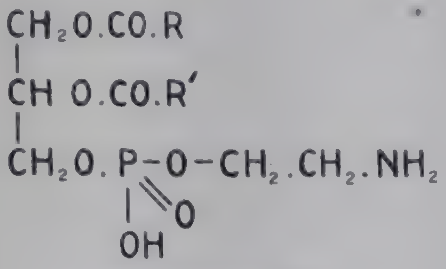
The presence of an acidic and basic group enables lecithin to combine with bases and acids.

Since hydrolysis of lecithins purified from various tissues gives more than two fatty acids it is concluded that the lecithins, like the true fats, are really mixtures of several similar lecithins. At least six fatty acids are commonly obtained by hydrolysis of lecithins ; these are palmitic, stearic, oleic, linoleic, linolenic and arachidonic ; other acids have also been found.

Even with the constituents specified above there are a large number of possible isomers. Referring back to the formulæ it will be seen that, theoretically, with two different fatty residues R, R′ there are two structural isomers of α-lecithin each giving two optical isomers, whereas there are two optical isomers for the β form—six in all. Most of the natural lecithins are dextrorotatory.

An interesting light has been thrown on the structure of lecithins by the action of cobra venom, which contains an enzyme which partially hydrolyses the lecithin, giving a *lysolecithin*. The enzyme is remarkably specific in that it only hydrolyses the groupings containing unsaturated fatty acids. (It does not act upon a completely saturated (synthetic) lecithin.) Since lysolecithins so far obtained always contain either palmitic or stearic acids and one OH of the glycerol free, it is concluded that natural unsaturated lecithins must be mixed lecithins, *i.e.*, they contain one molecule each of saturated and unsaturated fatty acid. Since, however, the unsaturated fatty acids far exceed the saturated acids in natural lecithins, the existence of completely unsaturated lecithins must be admitted.

Kephalins always occur associated with lecithins in the tissues and are very similar in properties. The distinction lies in the nature of the basic constituent which in the kephalins is **aminoethyl alcohol**, $NH_2.CH_2.CH_2.OH$.

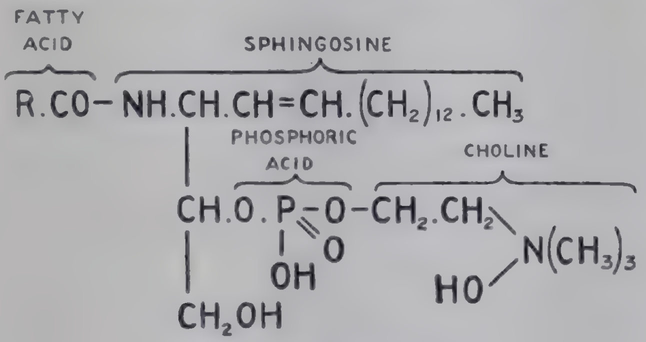


α-KEPHALIN

β-kephalins are also found. Cobra venom behaves as with lecithin, giving *lysokephalins*.

Howell's claim that the substance thrombokinase which initiates the clotting of blood is identical with kephalin is not convincing without further evidence.

Sphingomyelins. These compounds have properties more like the cerebrosides; they are stable to light and air, white and crystalline. The chemical distinction from lecithins lies in the absence of glycerol, which has been replaced by a base called **sphingosine**, $CH_3.(CH_2)_{12}.CH{=}CH.CH(NH_2).CH(OH).CH_2OH$, only found in animal tissues. The other constituents are the same as those of lecithin, namely, fatty acid, phosphoric acid and choline. The structure of these compounds is probably :—



SPHINGOMYELIN

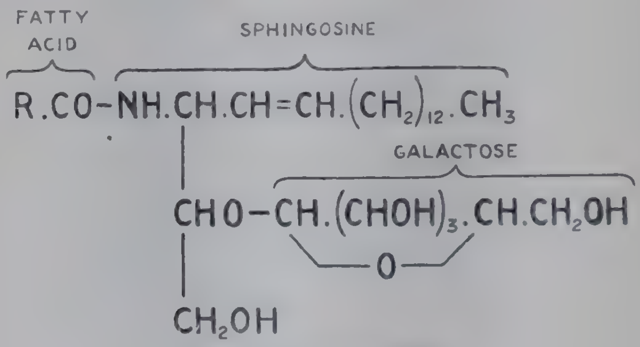
Note that four stereoisomers are possible, since there are two asymmetric carbon atoms. (The natural forms are dextro-rotatory.) Since three fatty acids (stearic, lignoceric and nervonic) have been obtained by hydrolysis of sphingomyelin preparations, it is probable that there are at least three sphingomyelins, not counting possible stereoisomers.

An interesting type of phosphatide in which the choline of lecithin is replaced by Ca has been recently found in plants. So far as is known this does not occur in animals.

2. Cerebrosides (Glycolipides)

Associated with phosphatides in most tissues, especially brain, are cerebrosides, often described more specifically as **galactolipides** (galactolipins), which in general properties resemble the sphingomyelins. Their characteristic is the presence of galactose and they can be regarded as glycosides. Three cerebrosides have been definitely identified, **Phrenosin, Kerasin, Nervon.** All are similar in structure, yielding on hydrolysis a fatty acid, sphingosine and galactose. The exact nature of the fatty acids is disputed; they may be mixtures of very closely related acids.

The probable formula is :—



CEREBROSIDE

The fatty acids are :—

d-Phrenosin .	Phrenosinic or cerebronic acid (C_{24}, OH)
l-Kerasin .	Lignoceric acid (C_{24})
l-Nervon .	Nervonic acid (C_{24}, 1 double bond).

The existence of an **oxynervon** has been deduced from the isolation of hydroxynervonic acid. The cerebrosides are particularly abundant in brain and nerves, where they form a considerable proportion of the myelin sheath. Biochemically they are particularly interesting, since both sugar and fatty acid occur in the same molecule, and the sugar is galactose, which is only found elsewhere in the body in combination with certain proteins and in lactose during lactation.

DERIVED LIPIDES

1. Fatty Acids (21)

It is assumed that the reader is acquainted with the general properties of fatty acids. Those commonly found in nature are listed below.

Fatty Acids occurring in Nature

(1) **Saturated Normal** (straight chain) **Acids**, $C_nH_{2n}O_2$.

Acid	Carbon Atoms	Acid	Carbon Atoms
Formic	1	Palmitic	16
Acetic	2	Stearic	18
Butyric	4	Arachidic	20
Caproic	6	Behenic	22
Caprylic	8	Lignoceric *	24
Capric	10	Cerotic	26
Lauric	12	Melissic	30
Myristic	14		

(2) **Unsaturated Acids.** $C_nH_{2(n-x)}O_2$ where x = number of double bonds.

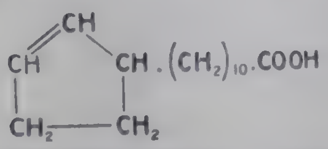
Acid	Carbon Atoms	Double Bonds
Oleic	18	1
Erucic	22	1
Nervonic	24	1
Linoleic	18	2
Elaeostearic	18	3
Linolenic	18	3
Arachidonic	20	4
Clupanodonic	22	5

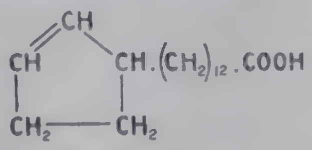
(3) **Hydroxyacids.**

Acid	Carbon Atoms	Double Bonds	—OH Groups
Ricinoleic	18	1	1
Cerebronic *	24	0	1
Hydroxynervonic *	24	1	1

* The structures of these acids are uncertain.

The natural fatty acids are normal straight chain acids *with an even number of carbon atoms*; acids with an odd number of carbons or branched chains are very rare. Two unique unsaturated fatty acids, used therapeutically in the treatment of leprosy, and found in *chaulmoogra oil*, have a five-carbon ring at the end of a straight chain; they are **hydnocarpic** and **chaulmoogric acids.**

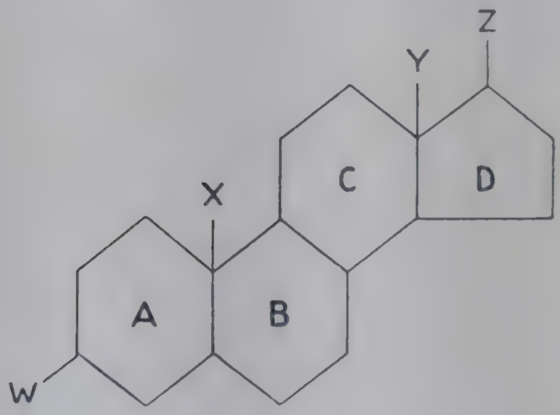

HYDNOCARPIC ACID


CHAULMOOGRIC ACID

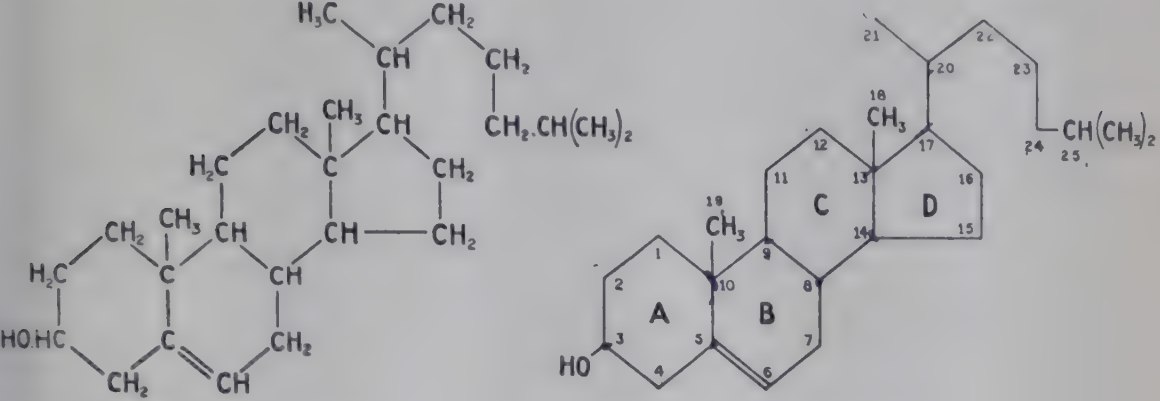
2. Sterols (4, 24)

Many members of this group have long been known. **Cholesterol,** one of the best known, was first found in human gall-stones, but is now known to be a universal constituent of the cells of higher animals. It is especially abundant in brain, nervous tissue and the suprarenal glands. Cholesterol forms about 17% of the solids of human brain. It is present in fair amount in liver, kidney and epidermis and found in all animal fats. Associated with cholesterol in the tissues is a saturated alcohol **dihydrocholesterol** ; a stereoisomer of dihydrocholesterol is found in fæces—**coprosterol.** In the tissues cholesterol is frequently present as esters.

Until a few years ago cholesterol was known to be present in many tissues, but was regarded as of more chemical than physiological interest. The last few years, however, have revealed that many compounds related to cholesterol not only occur in the body but have diverse and important physiological functions. The increased physiological interest in these compounds has led to intensive chemical investigation into their structure. These compounds include bile acids, vitamin D, and the male and female sex hormones. The essential structure common to all these compounds consists of four fused rings arranged thus :—



There is always oxygen in the form of a hydroxyl or ketone group at **W**, either the same or a side chain at **Z**, and a methyl group at **Y**. Rings **C** and **D** are saturated and remarkably stable. In the sterols, **W** is always OH and **Z** a side chain of several carbon atoms, and **X** and **Y** are occupied by methyl groups. The individual sterols differ in the side chain and the degree of saturation of rings **A** and **B**. The formula of cholesterol is :—


H₃C
CH₂
CH
CH₂
CH₂
CH₃
CH
CH₂.CH(CH₃)₂
H₂C
C
CH₂
CH₃
CH
CH
CH₂
H₂C
C
CH
HO.HC
C
CH₂
CH₂
CH
21
20
22
23
18
CH₃
17
CH(CH₃)₂
24
25
12
11
13
16
C
D
19
CH₃
14
15
1
9
2
10
8
A
B
3
5
7
HO
4
6

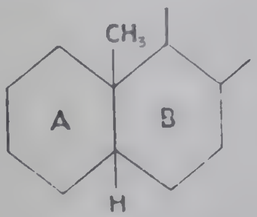
Full Formula

Shortened Formula with numbering

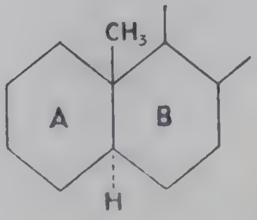
CHOLESTEROL

As the formula indicates, cholesterol and the other sterols are distinct from other lipides in that they cannot be hydrolysed by NaOH—they are *unsaponifiable*. Physically cholesterol is a well-defined white crystalline substance which is very stable. It melts at 149° C., much higher than other types of lipides. From alcohol it usually crystallises in very characteristic glistening rhombic plates with an irregular or broken corner. Its esters are strictly waxes.

It will be seen that cholesterol contains 7 asymmetric carbon atoms in the rings above. If the rings are completely saturated there are eight asymmetric carbon atoms (3, 5, 8, 9, 10, 13, 14, 17). This means that there are $2^8 = 256$ theoretically possible stereoisomers. Fortunately, in all known natural compounds containing this ring system, stereoisomerism is restricted to carbon atoms 3 and 5. There are thus only four types we need consider. Referring for the moment to the parent saturated hydrocarbon which loses asymmetry in position 3, there are two stereoisomers due to the asymmetry of carbon 5. The relative space positions of the H atoms (only rings **A** and **B** are shown) can be represented :—

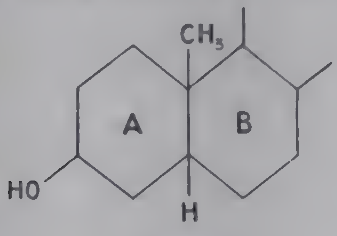


Normal Series

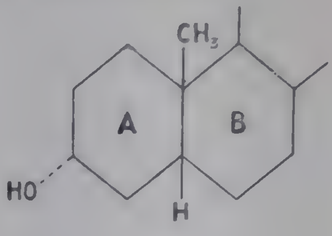


Allo-Series

These stereoisomers are described as belonging to *normal* and *allo* series. Now to return to the saturated alcohol corresponding to cholesterol and position 3 we have in the *normal* series :—



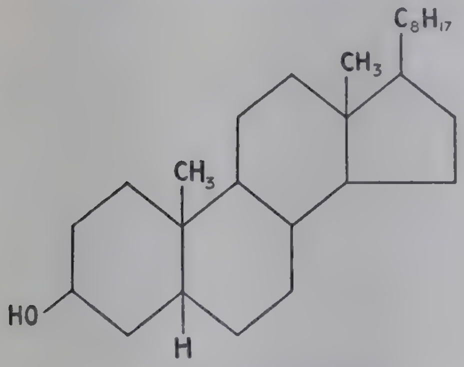
Normal



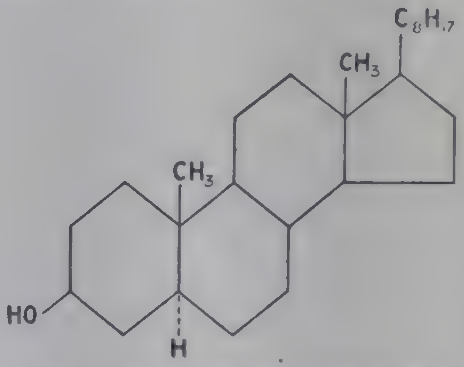
Epi-

Similarly there is a *normal* and *epi*-form of the *allo*-series.

Now coprosterol and dihydrocholesterol are two of the isomeric reduction products of cholesterol in which the double bond between atoms 5 and 6 has been eliminated. Coprosterol belongs to the *normal* series and dihydrocholesterol to the *normal allo*-series.

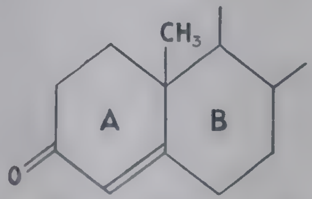


COPROSTEROL



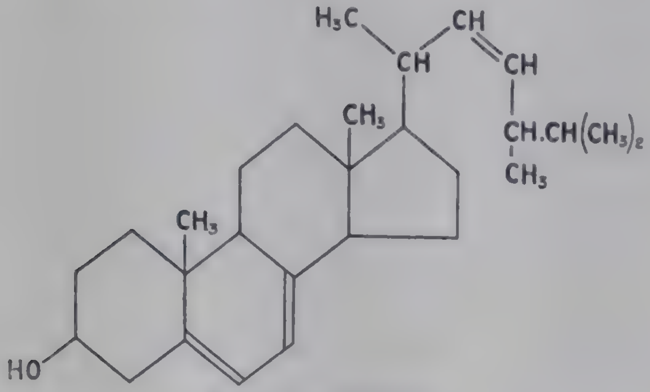
DIHYDROCHOLESTEROL

Epi-forms of both these sterols have been prepared. It will be seen that interconversion of the four stereoisomers is, at least theoretically, simple by obliterating the asymmetric carbon atoms and reforming them by reduction. Both atoms have asymmetry obliterated in the following compound :—



Suitable reductions of this compound would give the four stereoisomers. Cholesterol is the *normal* form and exists naturally as the lævorotatory compound.

Although cholesterol is found universally in animal tissues it is not found in plants, which contain a series of sterols called collectively the *phytosterols*. Of these the only one of direct interest in animal biochemistry is **ergosterol,** since this is a precursor of vitamin D_2. Ergosterol is less stable than cholesterol, having three double bonds.



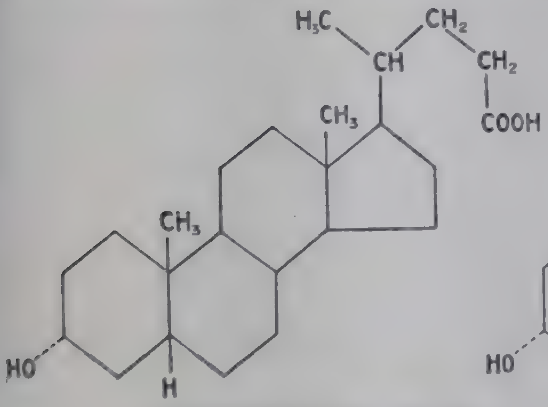
ERGOSTEROL

The so-called " **isocholesterol** " found in the form of esters of palmitic, stearic and oleic acids in wool-fat and human sebum is a mixture of the unsaturated *agnosterol* and a non-sterol compound which was mistakenly labelled as *lanosterol.* The nature of the latter, which predominates, has not been determined.

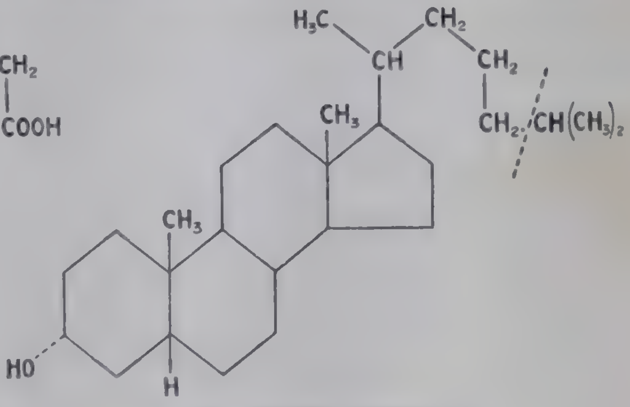
There is little doubt that cholesterol can be synthesised in the animal. How or where is unknown.

Relation of Cholesterol to the Bile Acids

The bile acids are *epi*-hydroxy derivatives of a saturated acid called **cholanic acid** (p. 197). The close relationship with the sterols is seen by comparing the formulæ of 3-monohydroxycholanic acid, which is one of the bile acids (lithocholic acid) and *epi*-coprosterol which belongs to the same stereochemical series.



LITHOCHOLIC ACID



Epi-COPROSTEROL

Oxidation of *epi*-coprosterol at the dotted line would give lithocholic acid. The natural coprosterol, however, belongs to the *normal*, not *epi*-, series, and is only formed by bacteria in the large intestine; the reduced form of cholesterol within the body is dihydrocholesterol, a member of the *allo*-series and not *epi*-dihydrocholesterol, so that direct conversion of dihydrocholesterol is improbable. Cholesterol has been converted to lithocholic acid in the laboratory, but there is as yet no proof that this reaction can occur *in vivo*.

Relation of cholesterol to hormones of adrenal cortex. See p. 344.

Relation of cholesterol to sex hormones, See pp. 339, 344.

Relation of cholesterol to vitamin D. See pp. 355.

CHAPTER VII

PROTEINS (1, 2, 4, 6, 9, 28, 80)

PROTEINS are not only essential and universal cell constituents, but are practically the sole form in which the body can replace lost nitrogen. Since the average well-fed man excretes about 15 g. of nitrogen per day, chiefly as urinary urea, it is obvious that proteins are important dietary constituents. We are also largely dependent on proteins for our supply of sulphur, for the body cannot utilise this element in inorganic form; most proteins contain sulphur in a form which we can readily use. Many proteins contain certain chemical groupings which are essential to our welfare, since we are unable to synthesise them in our bodies; in addition, proteins can be used for ordinary energy requirements just like sugar.

Apart from their importance as articles of diet, proteins in animals help to form supporting and protective structures, such as bones, cartilage, skin, nails and hair, and provide a large part of the total solids of the body; in plants carbohydrate predominates and provides these structures. The uses of proteins are not restricted to their *rôle* in the body or as food. Man uses proteins extensively for the manufacture of his clothes and shoes in the form of wool, silk, furs and leather as well as for his amusements; the musician and the tennis player are at one in their indebtedness to the protein of catgut.

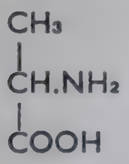
There are a large number of proteins with varying properties. All contain carbon, hydrogen, oxygen and nitrogen. Some have, in addition, sulphur, phosphorus or even other elements.

Proteins are of even greater complexity than polysaccharides and compound lipides, but, like them, can be hydrolysed to simple units (called amino-acids) by boiling with acids or alkalies, although not so easily. Since there are usually about twenty different amino-acids in any given protein, and there may be many molecules of each amino-acid (a protein of M.W. 35,200 would contain about 288 amino-acid molecules in each molecule of protein), it is clear that there are infinite ways in which a protein could be made up, even if we exclude the presence of the carbohydrate which is found in some proteins. We have, indeed, evidence, by isolation, of the existence of a vast array of proteins which can be distinguished by the chemical means at present available; but we have physiological evidence of even greater variation in that apparently identical proteins of two different species have profoundly different physiological action. One of the serum proteins of a rabbit repeatedly injected into another rabbit will produce no ill-effects. If, however, the (apparently) same protein of another species be injected, the second or later injection may be followed by a severe reaction which may even be fatal. No two proteins seem to be exactly alike in their physiological action. This physiological specificity is, indeed, one of the most characteristic features of the proteins as a group. The reason for this specificity is little understood.

Before proceeding further with the proteins we must study the simple units of which they are composed.

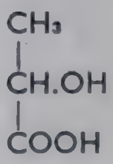
AMINO-ACIDS (28, 80)

These amino-acids can all be considered as derivatives of the simplest α-amino-acid, *aminoacetic acid*. But since the body has an extraordinary facility for dealing with molecules of 3 carbon atoms it is preferable to regard α-amino-propionic acid or **alanine**, $CH_3.CH(NH_2).COOH$, as the parent substance. Note the relationships between alanine and 3 carbon compounds derived from carbohydrates and fats.



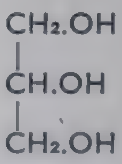
$$\begin{array}{l} \mathrm{CH_3} \\ | \\ \mathrm{CH.NH_2} \\ | \\ \mathrm{COOH} \end{array}$$

Alanine



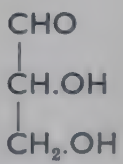
$$\begin{array}{l} \mathrm{CH_3} \\ | \\ \mathrm{CH.OH} \\ | \\ \mathrm{COOH} \end{array}$$

Lactic Acid



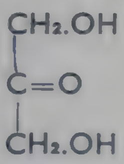
$$\begin{array}{l} \mathrm{CH_2.OH} \\ | \\ \mathrm{CH.OH} \\ | \\ \mathrm{CH_2.OH} \end{array}$$

Glycerol



$$\begin{array}{l} \mathrm{CHO} \\ | \\ \mathrm{CH.OH} \\ | \\ \mathrm{CH_2.OH} \end{array}$$

Glycerose



$$\begin{array}{l} \mathrm{CH_2.OH} \\ | \\ \mathrm{C{=}O} \\ | \\ \mathrm{CH_2.OH} \end{array}$$

Dihydroxyacetone

The body can readily replace the NH_2 of alanine by OH, giving lactic acid. If we refer back to p. 61, the relationship of the other compounds shown will be clear. Here, then, we have a connecting link between the three big classes of substances composing our bodies and food. The importance of these three carbon compounds will be seen when we come to study intermediary metabolism. It should be noted that all amino-acids from proteins are α-amino-acids, *i.e.*, NH_2 is attached to the C next to the carboxyl group.

Amino-acids can be classified in many ways. Since the separation of amino-acids from the hydrolysis products of a protein is based upon their division into three groups depending on their acidicity or basicity, we will adopt this as a method of classification for all amino-acids containing an alanine residue, and relegate to a fourth group two amino-acids in which the sole basic portion is in the form of a ring. An amino-acid like alanine having a basic NH_2 and an acid COOH group of equivalent strength will be approximately neutral. If, however, an amino-acid has an excess of either acidic or basic groups it will be predominantly acidic or basic in character. Thus we can divide amino-acids into Neutral, Acidic and Basic Amino-acids.

I. Neutral Amino-acids (*Monoaminomonocarboxylic acids*)

Glycine (*α-aminoacetic acid*)

$$H.CH(NH_2).COOH.$$

d-Alanine (*α-aminopropionic acid*)

$$CH_3.CH(NH_2).COOH.$$

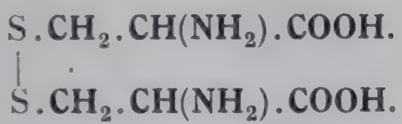
l-Serine (*β-hydroxy-α-aminopropionic acid*)

$$HO.CH_2.CH(NH_2).COOH.$$

l-Cysteine (*β-thiol-α-aminopropionic acid*)

$$HS.CH_2.CH(NH_2).COOH.$$

l-Cystine (*dicysteine*)



$$\begin{array}{l} S.CH_2.CH(NH_2).COOH. \\ | \\ S.CH_2.CH(NH_2).COOH. \end{array}$$

l-Threonine (*β-hydroxy-α-aminobutyric acid*)

$$CH_3.(HO)CH.CH(NH_2).COOH.$$

l-Methionine (*γ-methylthiol-α-aminobutyric acid*)

$$CH_3S.CH_2.CH_2.CH(NH_2).COOH.$$

d-Valine (*α-amino-isovaleric acid*)

$$(CH_3)_2.CH.CH(NH_2).COOH.$$

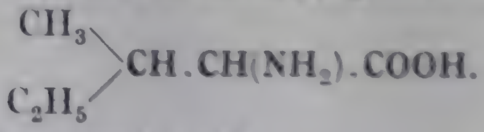
d-Norvaline (*α-aminovaleric acid*)

$$CH_3.CH_2.CH_2.CH(NH_2).COOH.$$

l-Leucine (*α-amino-isocaproic acid*)

$$(CH_3)_2.CH.CH_2.CH(NH_2).COOH.$$

d-Isoleucine (*β-methyl-β-ethyl-α-aminopropionic acid*)



$$\begin{matrix} CH_3 \\ C_2H_5 \end{matrix} \Big> \mathbf{CH.CH(NH_2).COOH.}$$

d-Norleucine (*α-aminocaproic acid*)

$$CH_3.CH_2.CH_2.\mathbf{CH_2.CH(NH_2).COOH.}$$

l-Phenylalanine (*β-phenyl-α-aminopropionic acid*)

$$C_6H_5.\mathbf{CH_2.CH(NH_2).COOH.}$$

l-Tyrosine (*β-p-hydroxyphenyl-α-aminopropionic acid*)

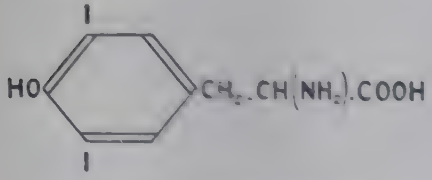
$$C_6H_4(OH).\mathbf{CH_2.CH(NH_2).COOH.}$$

l-Tryptophan (*β-indole-α-aminopropionic acid*)

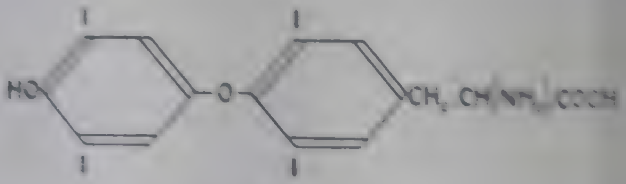
$$C_8H_6N.\mathbf{CH_2.CH(NH_2).COOH.}$$

In the formulæ in the table heavy type distinguishes the alanine residue from the rest of the molecule. Since alanine contains an asymmetric carbon atom, all these amino-acids (except glycine) can exist in optically active forms: the naturally occurring forms are indicated. The sulphur-containing acids, cysteine and cystine, are so readily interconverted that there is often some doubt as to which form actually occurs in the protein. Note the acids with aromatic rings.

Two other amino-acids, derivatives of tyrosine, should be included here, but as they are only found in special proteins they are not recorded in the main list.



3, 5-Diiodotyrosine



Thyroxine

Both are found in the thyroid gland. Several other amino-acids have been stated to occur, but their identity has not been satisfactorily established.

II. Acidic Amino-acids (*Monoaminodicarboxylic acids*)

l-Aspartic Acid (*α-aminosuccinic acid*)

$$HOOC.\mathbf{CH_2.CH(NH_2).COOH.}$$

d-Glutamic Acid (*α-aminoglutaric acid*)

$$HOOC.CH_2.\mathbf{CH_2.CH(NH_2).COOH.}$$

-Hydroxyglutamic Acid (*β-hydroxy-α-aminoglutaric acid*)
$HOOC.CH_2.(HO)CH.CH(NH_2).COOH.$

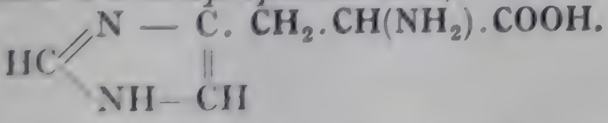
The alanine residue is indicated as in Group I. These amino-cids are especially abundant in the proteins of the seeds of cereals. hey may be present in proteins as their amides *asparagine* or *lutamine.*

III. Basic Amino-acids

l-Arginine (*δ-guanidino-α-aminovaleric acid*)
$NH=C(NH_2).NH.CH_2.CH_2.CH_2.CH(NH_2).COOH.$

l-Lysine (*α-, ε-diaminocaproic acid*)
$CH_2(NH_2).CH_2.CH_2.CH_2.CH(NH_2).COOH.$

-Histidine (*β-iminazole-α-aminopropionic acid*)

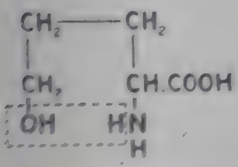


These acids are sometimes called the *hexone* bases. Two derivatives of arginine have been found amongst the decomposition products of proteins, **ornithine,** $NH_2.(CH_2)_3.CH(NH_2).COOH$, and **citrulline,** $NH_2.CO.NH.(CH_2)_3.CH(NH_2).COOH$, but it is doubtful whether they actually exist in the protein.

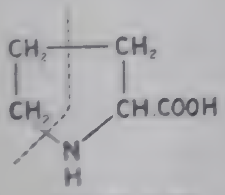
Group IV

l-Proline (*α-pyrrolidine-carboxylic acid*)
l-Hydroxyproline (*γ-hydroxy-α-pyrrolidine-carboxylic acid*)

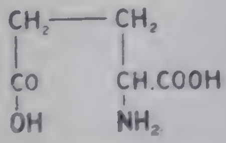
These acids are strictly not amino-acids as they contain no amino group. Since, however, proline could be formed from δ-hydroxy-α-aminovaleric acid by elimination of water the term is permissible; some maintain that proline does not exist in proteins but is formed in such a way on hydrolysis. The dotted



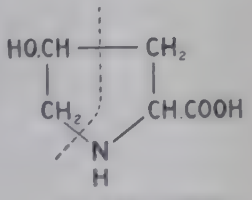
δ-Hydroxy-α-aminovaleric Acid



PROLINE



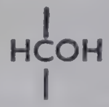
Glutamic Acid



HYDROXY-PROLINE

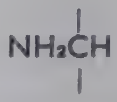
line marks the part of the molecule represented by alanine. Note that oxidative rupture of the ring of proline between the CH_2 and NH would give glutamic acid.

In all these acids the $=CH(NH_2)$ has the same spatial configuration which can be regarded as the opposite of that of the fifth carbon atom of *d*-glucose.



$$\text{H}\overset{|}{\underset{|}{\text{C}}}\text{OH}$$

Fifth Carbon *d*-Glucose



$$\text{NH}_2\overset{|}{\underset{|}{\text{C}}}\text{H}$$

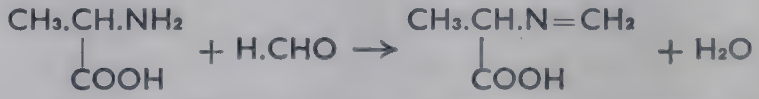
All α-amino Acids

Many of these amino-acids can be synthesised by the body; others apparently cannot. Young animals on a diet lacking certain amino-acids cease to grow and, if the supply is still withheld, die. For this reason these acids are referred to as *essential amino-acids*; they are listed on p. 392.

General Chemistry of Amino-acids

The amino-acids are colourless, well-crystallised compounds which readily form salts with both acids and bases. Some of the *copper* salts are particularly characteristic. Being organic acids, amino-acids also form *esters* with alcohols. Both the esters and copper salts have been used to separate the mixture of neutral and acidic amino-acids left after the basic amino-acids have been removed (usually as insoluble phosphotungstates) from a protein hydrolysate; the liquid esters are separated by fractional distillation *in vacuo* and the copper salts by means of various solvents.

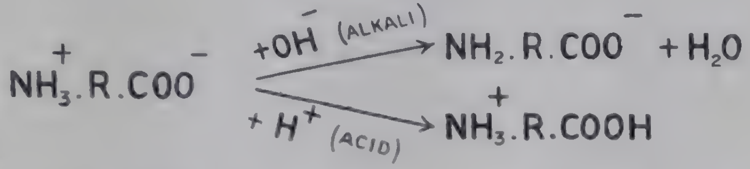
The amino group of an amino-acid can be converted into a neutral group by treating with formaldehyde, a methylene compound being formed :—



$$\underset{\text{COOH}}{\overset{\text{CH}_3.\text{CH}.\text{NH}_2}{|}} + \text{H.CHO} \rightarrow \underset{\text{COOH}}{\overset{\text{CH}_3.\text{CH}.\text{N}=\text{CH}_2}{|}} + \text{H}_2\text{O}$$

Alanine Formaldehyde α-Methyleneaminopropionic Acid

The resulting compound therefore behaves as an ordinary acid and can be titrated directly with alkali. After pretreatment with formaldehyde, therefore, it is possible to estimate the carboxyl groups * in an amino-acid (or protein) by simple titration—**formol titration.** This raises the question of the behaviour of amino-acids as electrolytes. Since they combine with both acids and alkalis they are called **amphoteric** electrolytes. A typical amino-acid $NH_2.R.COOH$ in solution ionises both as an acid and a base. When both types of ionisation occur to an equal extent, the molecule is electrically neutral and at its **isoelectric point.** Addition of acid or alkali will depress one type of ionisation so that the amino-acid will behave as a base or an acid. The ion at the isoelectric point which carries + and — charges internally neutralised is called a "**zwitterion.**" The three types of ions can be represented :



$$\overset{+}{N}H_3.R.CO\overset{-}{O} \xrightarrow{+O\bar{H}\ (\text{ALKALI})} NH_2.R.CO\overset{-}{O} + H_2O$$

$$\overset{+}{N}H_3.R.CO\overset{-}{O} \xrightarrow[(\text{ACID})]{+H^+} \overset{+}{N}H_3.R.COOH$$

The unionised salts of an amino-acid would be represented $HOOC.R.NH_2HCl$ (the hydrochloride) and $NH_2.R.COONa$ (sodium salt). When there are excess $—NH_2$ or $—COOH$ groups in the acid (*e.g.*, lysine or aspartic acid) it can be assumed that these are free and not zwitterions.

PROTEINS

The Structure of Proteins (2, 6, 10, 31, 80, 87)

The simplest way in which amino-acids could join together to form a protein would be by successive coupling of amino and carboxyl groups thus :—

$$\underset{\text{Glycine}}{NH_2.CH_2.CO\,OH} + \underset{\text{Alanine}}{H\,NH.CH(CH_3).COOH} \longrightarrow \underset{\text{Glycylalanine}}{NH_2.CH_2.CO.NH.CH(CH_3).COOH} + H_2O$$

This compound is called a dipeptide because it contains two

---

* To be accurate, it is amino groups which are estimated, not carboxyl. An amino-acid exists as a " zwitterion " which does not react with formaldehyde. Addition of NaOH causes the following reaction :—

$$Na^+ + OH^- + NH_3^+.R.COO^- \longrightarrow H_2O + NH_2.R.COO^- + Na^+.$$

Formaldehyde then reacts with the amino group. Thus the amount of NaOH required for titration is really a measure of the amino groups.

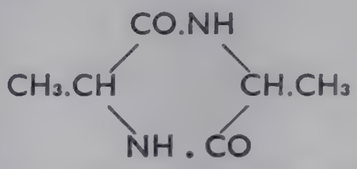
amino-acids joined by a "peptide" link, —CO—NH—. With more amino-acids we get tripeptides, *e.g.* :—

$$NH_2.CH_2.CO.NH.CH(CH_3).CO.NH.CH_2.COOH$$

Glycylalanylglycine

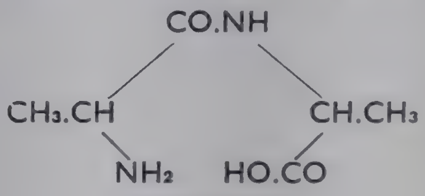
tetrapeptides, and so on; a chain of many amino-acids is a polypeptide. Amino-acids cannot be joined together in this way directly in the laboratory.

If the ester of an amino-acid is heated it forms an anhydride from 2 molecules of amino-acid thus :—



Alanine Anhydride or Diketopiperazine

These anhydrides are called diketopiperazines from the name of the parent ring. If a diketopiperazine is heated with hydrochloric acid the ring first breaks, giving a dipeptide thus :—



Alanylalanine

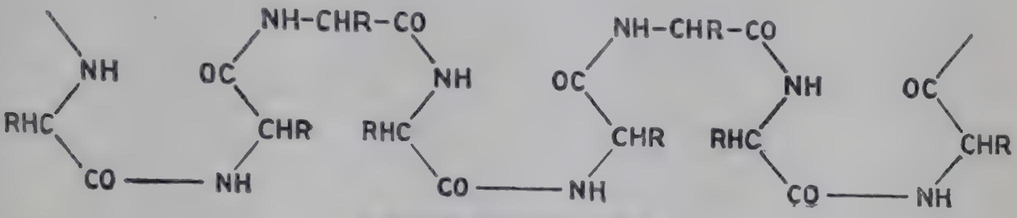
The fact that proteins which on hydrolysis yield amino-acids have relatively few *free* amino or carboxyl groups makes it almost certain that the amino-acids are linked mainly in peptide (or diketopiperazine) form. Both peptides and diketopiperazines can be obtained by careful hydrolysis of proteins. The existence of diketopiperazine and other ring structures in proteins has been suggested,* but the sum of the evidence leaves little doubt that the linking of amino-acids in proteins is predominantly of the peptide type.

Now proteins have very large molecular weights, and yet, as a class, have properties sufficiently characteristic to suggest that the amino-acids are arranged, not in a haphazard manner, but in a pattern which is, basically, common to all. Further, many

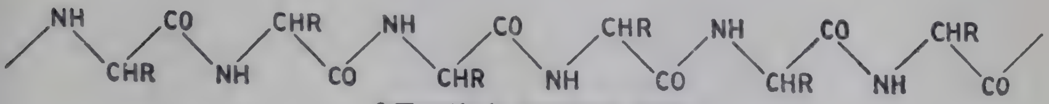
* See Refs. 2, 10, 31, 80.

proteins form well-defined crystals. Recently evidence of such a pattern has been obtained.

X-rays and other physical measurements reveal the existence of two types of protein, a fibrous one with elongated rod-like molecules and a type with globular molecules. As an example of the fibrous type, that is, of proteins which exist as fibres, let us take the keratin of which hair is fundamentally composed. On stretching, the α-keratin of normal hair passes into β-keratin, α-keratin reforming when the tension is released. If, however, the stretched hair is subjected to steam, as in the process of "permanent waving," it loses its power of recovery and becomes "set" as β-keratin. The X-ray photographs suggest that these protein molecules consist of thin bundles of polypeptide chains which lie parallel to the fibre axis and which Astbury has represented :—



α-Keratin in Ordinary Hair

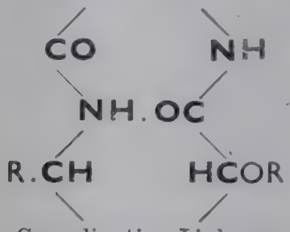


β-Keratin in Stretched Hair

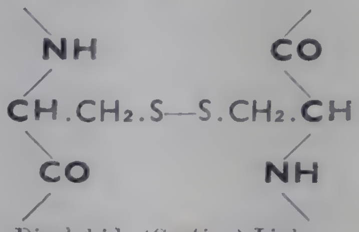
If a polypeptide chain is formed in this way, all the R groups (R = an amino-acid less $-CH(NH_2)COOH$) project on the same surface of the molecule. Examples of proteins of the fibrous type include collagens, keratins, fibrin, fibroin and myosin.

In the globular type of protein the polypeptide chains are not parallel and the molecule has a globular or ellipsoidal shape. (Very few proteins have been found which are spherical). Examples of this type include soluble native proteins such as albumins, globulins, insulin, myoglobin, pepsin, trypsin and zein. They can assume fibrous form on denaturation (p. 104).

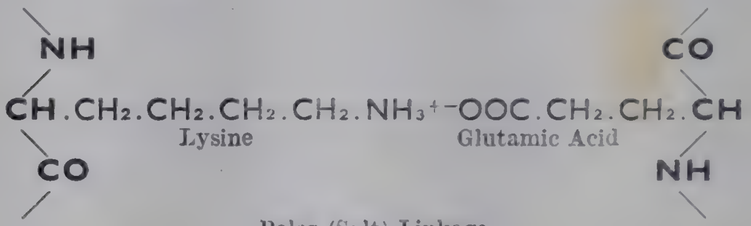
The way in which polypeptide chains are linked together is not fully established. The position, nature and number of the linkages are important in determining the configuration and the stability of the protein. Several types of bridge linkages are probable including co-ordination linkages, disulphide or cystine (–S–S–) linkages and polar or salt linkages between amino or carboxyl groups. The ε-amino group of lysine and the β- and γ-carboxyl groups of aspartic and glutamic acids are not engaged in peptide linkages and, therefore, can form polar linkages These types of linkages are represented below, the main polypeptide chains (" backbone ") being printed in heavy type.



Co-ordination Linkage



Disulphide (Cystine) Linkage



Polar (Salt) Linkage

The stability of the bridge linkages differs in the two types of proteins. In the fibrous form the bridge linkages are fairly stable but in the globular form they are usually labile so that the polypeptide chains may readily be re-orientated. Oxidation of a protein may cause linkage between the –SH of cysteine units of polypeptide chains by the formation of the disulphide cystine (–S–S–); this linkage is readily broken by reducing agents or by alkali. The state of these linkages has an important bearing upon the properties of the protein. For example, insulin and some anterior pituitary hormones are only physiologically active when in the disulphide (–S–S–) form; some proteolytic enzymes are active in the –SH form, others in the disulphide form. This may, in part, explain the effect of pH upon the activity of these enzymes.

Recent chemical analyses of proteins suggest the arrangement of particular amino-acids in a repeating pattern. The molecular ratios of basic amino-acids appear to be characteristic for any one type of protein. Some typical ratios are given in the table on the next page.

It has been claimed that glycine, proline, hydroxyproline, arginine, alanine, leucine (+ isoleucine) and lysine occur in gelatin

MOLECULAR RATIOS OF AMINO-ACIDS IN PROTEINS

Protein.	Arginine.	Histidine.	Lysine.
Albumin (egg)	3	1	3
Fibrin	8	3	12
Gelatin	4	—	3
Hæmoglobin	3	8	9
Keratin	12	1	4
Orosin (p. 158)	10	—	18

in the molecular proportions 24 : 12 : 8 : 4 : 8 : 4 : 3, which suggests a periodicity of 3, 6, 9, 18, 9, 18, 24, *i.e.*, every third amino-acid in the peptide chain is glycine, every sixth proline, and so on. A similar periodicity has been claimed for egg albumin and fibrin for at least eight amino-acids. This conception is not, however, supported by evidence from X-ray studies or enzymic fission of proteins. Many workers consider that analytical methods are not yet sufficiently accurate as to warrant such definite conclusions.

Molecular Weights of Proteins (31, 80)

The enormous size of a protein molecule makes the determination of molecular weight by physical methods such as osmotic pressure measurement a matter of great difficulty, which is not a little enhanced by the preliminary difficulty of getting the protein in a state of purity. Even minimal molecular weights, calculated from analytical data such as the amount of one particular amino-acid (*e.g.*, cystine) or atom (*e.g.*, iron) on the assumption that only one molecule or atom is present, indicate very high values, *e.g.*, 17,000 for hæmoglobin. The physical methods indicate still higher values. Recently Svedberg has applied the measurement of sedimentation velocity and equilibrium in an ultracentrifuge with remarkable results, which suggest that proteins fall into groups, in each of which the molecular weight approximates to a simple multiple of 17,600 (equivalent to approximately 144 amino-acid units). Proteins are known with molecular weights approximately 1, 2, 4, 8, 16, 24, 48, 96, 168, 192, and 384 times 17,600. Most proteins fall into the first five groups. The values of 24 × 17,600 and over have been obtained from the respiratory pigments of lower animals. Even higher values are given by the virus proteins (10 to 8,500 millions). Some examples of values found in Svedberg's laboratory are given in the table.

MOLECULAR WEIGHTS OF PROTEINS

PROTEIN	M.W. by Sedimentation Velocity	M.W. by Sedimentation Equilibrium	M.W. approximates to
Lactalbumin	17,500	—	1 × 17,600
Myoglobin	17,200	17,500	
Zein	35,000	—	2 × 17,600
Lactoglobulin	41,800	37,900	
Ovalbumin	43,800	40,500	
Insulin	40,900	35,100	
Pepsin	35,500	39,200	
Carboxyhæmoglobin (horse)	69,000	68,000	4 × 17,600
Serum albumin (horse)	70,200	66,900	
Serum globulin (horse)	167,000	150,000	8 × 17,600
Edestin	309,000	—	16 × 17,600
Hæmocyanin (*Palinurus*)	446,000	447,000	24 × 17,600
Hæmocyanin (*Helix*)	6,630,000	6,680,000	384 × 17,600

Not all proteins, however, have molecular weights which fit into Svedberg's groups, *e.g.*, gliadin and papain 27,000, the yellow enzyme 82,000, human anti-pneumococcus serum globulin 195,000, catalase 248,000 and urease 480,000. The number of such proteins is large enough to justify the query whether Svedberg's unit may be just a coincidence.

Svedberg's investigations have revealed the interesting fact that proteins in solution may very easily dissociate (*e.g.*, by a change in protein concentration, or in pH, or in salt concentration) into components of lower molecular weight which may re-associate on restoring the original conditions. This suggests that the proteins of higher molecular weights are built up by aggregation of definite units.

The foregoing does not apply to protamines and histones which are very much simpler than other proteins and may have molecular weights as low as 2,000.

General Properties of Proteins

In view of their high molecular weight, proteins can only form colloidal solutions. They are not soluble in any of the fat solvents; a few plant and other proteins are soluble in 70% alcohol. Different proteins have varying solubilities in water and salt solutions, a fact employed in their separation. They combine with heavy metals or acids of high molecular weight to form insoluble precipitates of uncertain composition. Protein properties and reactions are dependent to a large extent on the nature and arrangement of the amino-acids. That there is both qualitative and quantitative variation in the amino-acids of proteins is clear from the table on p. 102, in which the composition of a few well-known proteins is listed. Among the points in this table which should be noted are :—

(1) The relative absence of *glycine* from many proteins, of *cystine*, *tryptophan* and *tyrosine* from gelatin, and of *lysine* and *tryptophan* from zein.

(2) The high proportion of *cystine* in keratin, of *glutamic acid* in many proteins, especially the plant proteins gliadin, glutelin, and zein, of *arginine* in salmine, of *proline* in gelatin and gliadin, of *histidine* in globin, of *glycine* in gelatin.

(3) The totals. The methods of analysis of proteins are very difficult and some decomposition may occur during hydrolysis. Most of the proteins given in the table have been fairly extensively analysed. Others which have not been examined so carefully may have totals of under 50%. Very few proteins have been fully analysed. It must be realised that the totals should exceed 100%, for water is added on during hydrolysis.

(4) Some proteins contain a small proportion of *carbohydrate*.

Now, although many of the amino and carboxyl groups of the constituent amino-acids have been neutralised in the formation of peptide linkages, there will always be some free groups, either from the ends of peptide chains, or the excess groups of acidic or basic amino-acids. Proteins, in fact, show many of the amphoteric properties of amino-acids. Those composed of a high proportion of basic amino-acids, *e.g.*, protamines, are predominantly basic in

COMPOSITION OF PROTEINS

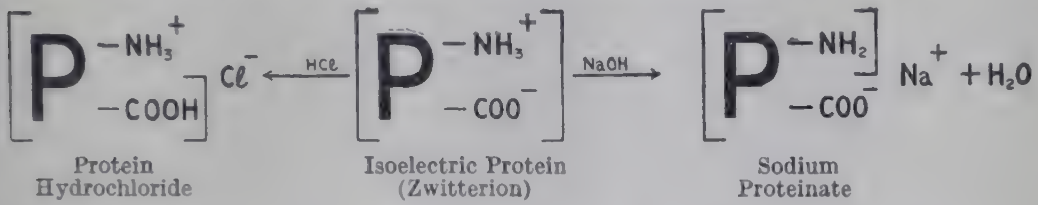
	Salmine.	Globin (Human).	Globin (Horse).	Lact-albumin.	Ovalbumin.	Blood Fibrin.	Edestin (Globulin).	Gliadin (Wheat).	Glutelin (Wheat).	Zein (Maize Gliadin).	Human Hair Keratin.	Horse Hair Keratin.	Wool Keratin.	Ox Horn Keratin.	Gelatin.	Caseinogen.	Vitellin.
Glycine				0·4	0·0		3·8	0·0	0·9	0·0		4·7	0·6	0·1	25·5	0·4	0·0
Alanine	+		4·2	2·5	8·4		3·6	2·0	4·7	9·8		4·7	4·4	2·2	8·7	1·8	0·8
Serine	7·8		0·6	1·8			0·3	0·1	0·7	1·0		0·6	0·1	1·0	0·4	0·5	
Cystine		0·8	0·3	4·0	2·3	1·6	1·8	2·7	0·0	1·6	16·5	8·0	10·0	7·2	0·1	0·7	
Threonine						+											
Methionine				2·6	4·6	2·6	2·1	2·0		2·3					1·0	3·4	
Valine	4·3			3·3	2·5		0·2	3·3	0·2	1·9		0·9	4·5		0·0	7·9	1·9
Norvaline														2·0		1·0	
Leucines *	+		29·0	19·4	15·2	+	20·9	6·6	6·0	25·0		7·1	11·5	20·6	7·1	9·7	9·9
Phenylalanine			4·2	2·4	5·2		3·1	2·3	2·0	6·6			0·0	0·5	1·4	3·9	2·6
Tyrosine		2·8	1·3	1·9	4·0		4·5	3·1	4·3	5·9		3·2	4·8	5·3	0·0	6·5	3·4
Tryptophan		2·3	+	2·7	1·3	5·0	1·5	0·8		0·2			1·8		0·0	2·2	
Aspartic acid			4·4	9·3	6·2	5·9	10·2	0·8	0·9	1·8		0·3	2·3	5·2	3·4	4·1	2·2
Glutamic acid			1·7	12·9	14·0	14·1	19·2	43·7	23·4	31·3		3·7	12·9	14·7	5·8	21·8	13·0
Hydroxyglutamic acid				10·0	1·4			2·4		2·5					0·0	10·5	
Arginine	87·4	3·5	5·4	3·2	6·0	7·7	15·8	3·2	4·7	1·8	8·0	7·6	10·2	2·0	9·1	5·2	7·9
Lysine	0·0	9·2	4·3	8·4	5·0	10·1	2·2	0·6	1·9	0·0	2·5	1·1	2·8	4·0	5·9	7·6	5·4
Histidine	0·0	8·6	11·0	1·5	2·3	2·5	2·1	2·1	1·8	1·2	0·5	0·6	0·7	2·3	0·9	2·6	1·2
Proline	11·0		2·3	4·0	4·2	5·1	4·1	13·2	4·2	9·0		3·4	4·4	3·6	19·7	8·0	4·2
Hydroxyproline			1·0				2·0			0·0					14·4	0·2	
Galactose				0·4												0·3	
Mannose					1·7			0·2									
Total	110·5	27·2	69·7	90·7	84·3	54·6	97·4	89·1	55·7	101·9	27·5	45·9	71·0	70·7	103·4	98·3	52·5

* Leucine, Isoleucine and Norleucine, if present.

This table has been compiled from many sources. The values represent what appear to be the best values for the amino-acids concerned and are not in all cases the amounts which have been isolated from the hydrolysed protein. For a recent compilation of values which have been obtained by isolation see Plimmer, Ref. 4.

character; acidic proteins, *e.g.*, gliadins, are likewise known; but whatever the composition, a protein can react both as an acid and a base, forming salts in each sense. Under suitable conditions proteins can even be quantitatively titrated with acid or alkali.

The behaviour of the protein is controlled by the pH of the medium in which it exists. This can be shown in a simple experiment with powdered gelatin which will only combine with silver ions on the alkaline side of pH 4·8, and ferrocyanide ions on the acid side of pH 4·7. At pH 4·75 it will react with neither anions nor kations. pH 4·75 is, in fact, the **isoelectric point** of gelatin. In solution, proteins behave very much as if they were amino-acids and ionise in the same way. The "**zwitterion**" and the respective salts with HCl and NaOH can be represented :—


$\left[P \begin{smallmatrix} -NH_3^+ \\ -COOH \end{smallmatrix}\right] Cl^-$
HCl
$\left[P \begin{smallmatrix} -NH_3^+ \\ -COO^- \end{smallmatrix}\right]$
NaOH
$\left[P \begin{smallmatrix} -NH_2 \\ -COO^- \end{smallmatrix}\right] Na^+ + H_2O$
Protein Hydrochloride
Isoelectric Protein (Zwitterion)
Sodium Proteinate

In thus ionising and behaving as amphoteric electrolytes, proteins are distinct from other colloids, *e.g.*, polysaccharides. Proteins must be regarded as being at the same time both colloids and crystalloids; the high molecular weight, which confers the colloidal properties, obliterates some typical crystalloidal properties such as diffusibility through membranes.

At the isoelectric point, not only the acid and base binding properties, but many physical properties of a protein are *minimal*, *e.g.*, the viscosity, swelling by imbibition of water, osmotic pressure, and, perhaps most important, its stability as an emulsoid colloid. *The tendency for a protein to coagulate or precipitate is greatest at its isoelectric point*; in other words, its solubility is minimal. This is a fact of great practical importance not only in testing for proteins (*e.g.*, albumin by the boiling test) but in the isolation of individual proteins (*e.g.*, insulin) from tissues.

Another property of proteins more characteristic of crystalloids rather than colloids is their formation of well-defined crystals. Egg albumin, horse serum albumin, insulin, hæmoglobins, hæmocyanins and the plant proteins edestin and excelsin can be crystallised comparatively easily.

Denaturation

If a protein such as albumin is precipitated from solution by alcohol, the precipitate is soluble in water if tested immediately; but if the precipitate is left in contact with the alcohol for half an hour it is no longer soluble in water. A change has taken place and the albumin is said to be "*denatured.*" This change, which is peculiar to certain proteins, may be promoted in a solution of a protein in several other ways, such as by heating, exposure to ultra-violet light, vigorous shaking, addition of acid, alkali, acetone, urea, etc. In the cold and in the region of its isoelectric point, a protein (if the solution is sterile) will remain almost indefinitely in its original or "*native*" form, but the process of denaturation is greatly accelerated by raising the temperature (about 600% for every 10° C. rise). Denaturation of some proteins is reversible.

The most perceptible change when a protein is denatured is diminished solubility, particularly at the isoelectric point. A soluble native protein of the albumin or globulin class becomes insoluble at its isoelectric point. When a protein is precipitated in this way it is said to be *coagulated* (see p. 107). This process is irreversible. Removed from the isoelectric point the change in solubility is not so marked.

X-ray studies have suggested that denaturation of a globular protein results in the loss of its specific configuration by re-orientation of the polypeptide chains to form a structure resembling a fibrous protein. Crystalline protein enzymes and certain hormones such as insulin are inactivated on denaturation. Denatured proteins are difficult to crystallise. Denaturation may not involve any change in molecular weight.

The Hydrolysis Products of Proteins

Whilst proteins are hydrolysed ultimately to amino-acids, several intermediate products can be recognised, especially if enzymes be used as the hydrolytic agents. These are **metaproteins, proteoses, peptones** and **polypeptides.** In the formation of metaproteins, the most complex of these products, only comparatively small changes have taken place in the molecule. The change occurs if the protein is left in 0·1% of HCl or NaOH at 37° C. for 24 hours, or more quickly in stronger solutions.

According as acid or alkali is used, metaproteins with slightly different properties, described as **acid** or **alkali-metaproteins**, are formed. The metaproteins differ in solubility from native proteins. Thus those from albumins are insoluble in water, though soluble in dilute acids and alkalis. Metaproteins of coagulable proteins are not heat coagulable. The exact change which occurs is unknown. In some instances a slight loss of ammonia and sulphur has been observed ; also when proteins are treated with alkali certain amino-acids are racemised. It will be realised from the foregoing that manipulation of proteins experimentally is liable to change their properties, a fact which must be borne in mind when interpreting results obtained.

Proteoses. The next simpler hydrolysis products are called proteoses. They are formed by the rupture of the protein molecule into several large complexes whose exact nature is unknown. Proteoses have many of the properties of the original protein, but are more soluble. They can be precipitated by strong salt solutions.

Peptones. Peptones chiefly differ from the proteoses in not being precipitated by saturated salt solutions ; another distinction is that the colour given in the biuret reaction with NaOH and $CuSO_4$ is pink rather than the usual purple. Protein colour reactions are usually given with greater difficulty ; peptones are not precipitated by all reagents which precipitate the protein. They can be regarded as complex polypeptides.

The stages in the hydrolysis of a protein can be represented :—

$$\begin{array}{l}\left\{\begin{array}{l}\text{Protein}\\ \text{Metaprotein}\\ \text{Proteose}\\ \text{Peptone}\end{array}\right.\\ \quad\text{Polypeptides}\\ \quad\text{Amino-acids.}\end{array}$$

The products covered by the bracket all give a biuret reaction and are still relatively large molecules. The simpler peptides and amino-acids which give no biuret reaction are often called abiuret products.

Classification of Proteins

In view of the great complexity produced from relatively few simple units it is hardly surprising that proteins are not readily distinguished by chemical means. A complete analysis of the amino-acid constituents is too time-consuming to be of practical value in this respect. In some proteins we have a readily identifiable component other than amino-acids, but in the majority we have to rely upon physical properties such as solubility in order to distinguish between different types. In this way we can distinguish nine groups :—

SIMPLE PROTEINS
(1) Protamines.
(2) Histones.
(3) (*a*) Albumins.
    (*b*) Globulins.
(4) (*a*) Gliadins.
    (*b*) Glutelins.
(5) Scleroproteins.

CONJUGATED PROTEINS	(PROSTHETIC GROUP)
(6) Phosphoproteins	(Phosphoric acid).
(7) Glycoproteins	(Carbohydrate).
(8) Nucleoproteins	(Nucleic acid)
(9) Chromoproteins	(Pigment).

The conjugated proteins can be regarded as ordinary proteins combined with another substance which forms what is called the *prosthetic group*. The characteristic properties of each group are recorded below ; individual members of the groups are described elsewhere.

Simple Proteins

(1) Protamines (26). These are undoubtedly the simplest proteins known, containing only about eight instead of twenty different amino-acids. There is no sulphur, phosphorus or aromatic amino-acid. Their molecular weights are abnormally small and may be as low as 2,000. The chief chemical characteristic is a predominance of arginine (*e.g.*, salmine contains over 80%) which makes them strongly basic to the extent of readily absorbing CO_2 from the air. They are soluble in water. They are found in association with nucleic acid in the ripe testicles of certain fish, *e.g.*, salmon (salmine) and herring (clupeine), and in chromosomes.

(2) Histones (26). The histones are simpler than the other proteins to be mentioned, but more complex than the protamines. They are intermediate, both in amino-acid content and basicity, between the protamines and proteins such as albumins or globulins. They are regarded by some as compounds of protamine with protein. They are soluble in water but precipitated by ammonia. They occur associated with nucleic acid in avian red blood corpuscles and mammalian lymphoid tissue, especially the thymus, and also in the spermatozoa of fish. The protein part of hæmoglobin, **globin**, is an atypical histone, having a predominance of histidine and lysine instead of arginine.

(3) **Albumins and Globulins (Coagulable Proteins).** These two groups can be regarded as typical proteins. They contain most of the amino-acids more or less uniformly distributed, and are neither markedly basic nor acidic. The chief distinction between the two groups lies in their solubility in pure water. Albumins are soluble, globulins are not, but both are soluble in dilute salt solutions. Many albumins are deficient in glycine. The characteristic property of albumins and globulins is that of being coagulated by heat. This occurs in most proteins of this class when their solutions are raised to about 75° C., although it is usual, when testing, to boil the solution. In relatively dilute solutions coagulation only occurs close to the isoelectric point and can be regarded as a heat denaturation, followed by the separation of the insoluble denatured protein from solution. It must be emphasised that the denatured protein is only insoluble close to the isoelectric point.

The term coagulation should be restricted when proteins are being considered, to describing the formation of an insoluble protein from a soluble one, as above, or in the clotting of blood or milk. When a protein is thrown out of solution, either as an insoluble salt or complex, or by changing its environment, and without interference with the protein molecule itself, other terms such as precipitation are preferable.

Albumins and globulins are usually precipitated from solution by treating with strong salt solutions of varying strength. Globulins, but not albumins, are insoluble in saturated magnesium sulphate, saturated sodium chloride and half-saturated ammonium sulphate solutions. Both are insoluble in saturated ammonium sulphate. The two groups can be separated on this basis. The precipitation of a protein by addition of strong solutions does not involve any chemical combination. The process is really one of dehydration, the strong salt solution removing osmotically the water shell (see p. 43) of the emulsoid and converting it to an unstable suspensoid which separates out. The precipitation is therefore more accurately described as "*salting out.*"

Albumins and globulins are most frequently found together as in serum, muscle, milk, and egg white. They are also widely distributed in plants, especially in the seeds and fruits. Many have been obtained in crystalline form.

Recent work has shown that many members of this group, even when highly purified, contain from 0·5 to 2% of carbohydrate which is probably galactose or mannose.

(4) **Gliadins and Glutelins.** Both types in this group are peculiar to the seeds of cereals. Although not animal proteins they are of importance as components of our food. Both types are insoluble in water but soluble in very dilute alkalis and acids. Gliadins can be separated from glutelins by means of 50–70% alcohol in which the former only are soluble. (Both are insoluble in absolute alcohol.) A mixture in approximately equal proportions is known as **gluten.**

Gliadin forms with water a sticky mass which gave origin to the term glutinous. This mass in flour binds together the particles of glutelin, starch, etc., to give the dough which is so important in bread-making (p. 425). The gliadin in flour is largely responsible for the adhesive properties of flour paste.

Gliadins and glutelins contain an exceptionally large amount of glutamic acid, especially the former; on the basis of their remarkably high proline content gliadins are also called *prolamins.*

(5) **Sclero proteins (Albuminoids).** These proteins are essentially similar to albumins and globulins (hence the name albuminoids), but are characterised by great stability and insolubility in water and salt solutions. They form most of the supporting structures of animals, *e.g.*, **collagen** in cartilage and white fibres of connective tissue, **elastin** in the yellow or elastic fibres, **ossein** in bones and teeth, **keratins** in horn, hair, wool, real silk (artificial silk is cellulose) and feathers. The keratins differ from the collagen-like members of this group in having an abnormally high sulphur content, chiefly in the form of cystine. They are peculiar in being readily attacked and dissolved by alkali sulphides, a fact finding application in the manufacture of hair-removing preparations.

By treatment with tannic acid, alum or various metal salts, the collagen of animal hides can be preserved in flexible condition giving the substance known as leather. By long boiling with water or treatment with steam collagen is partly hydrolysed, giving a soluble product known as **gelatin,** which can be regarded as a proteose of collagen. Gelatin solutions stronger than 1% have the power of forming heat reversible gels. The glues obtained by boiling down various residues of epithelial and skeletal tissue from the slaughter-house consist largely of gelatins.

Conjugated Proteins

(6) **Phosphoproteins.** These can be defined as proteins, containing phosphoric acid in organic combination, nucleoproteins being relegated to a separate class. The important members of this group, **caseinogen** found in milk and **vitellin** * found in birds' eggs, contain about 1% of phosphorus. Similar compounds are stated to be present in fish eggs. They are sparingly soluble in water and very dilute acid in the cold, but readily soluble in very dilute alkali. The phosphoric acid is liberated from organic combination by warming with NaOH and can only be detected by ammonium molybdate after it has been split off by this or some similar method.

(7) **Glycoproteins (Mucoproteins)** (27). These can be defined as proteins containing carbohydrate as the prosthetic group, nucleoproteins again being excepted. Glycoproteins, on boiling with dilute acids, readily yield reducing carbohydrate and often inorganic sulphate. The prosthetic group is frequently a sulphuric acid ester of a polysaccharide composed of hexosamine and glucuronic acid units. The hexosamine units are also acetylated, probably on the amino group. Two such polysaccharides are known, namely **mucoitin** and **chondroitin,** but their constitution has not been definitely elucidated. The hexosamines are glucosamine and chondrosamine respectively. The former is more widely distributed. The complete prosthetic groups are known as **mucoitin** and **chondroitin sulphuric acids.**† Proteins of this type can be divided into two classes :—

Mucins, which give slimy or gummy solutions, and are precipitated from solution by acetic acid in sticky strands. Many function as lubricants.

Mucoids, which give solutions which may be viscous, but are non-slimy. They are precipitated from solution by alcohol as white powders. They are not precipitated by acetic acid. They form the groundwork of supporting tissues. Both are soluble in dilute alkalis.

Mucins are found in saliva, mucous membrane of respiratory passages, and vitreous humour, and mucoids in cartilage, tendons, bone, serum, umbilical cord, etc. Mucoitin sulphuric acid has been found in both mucins and mucoids; chondroitin sulphuric acid has only been found in mucoids. Some mucoids, however, do not contain sulphuric acid or uronic acid.

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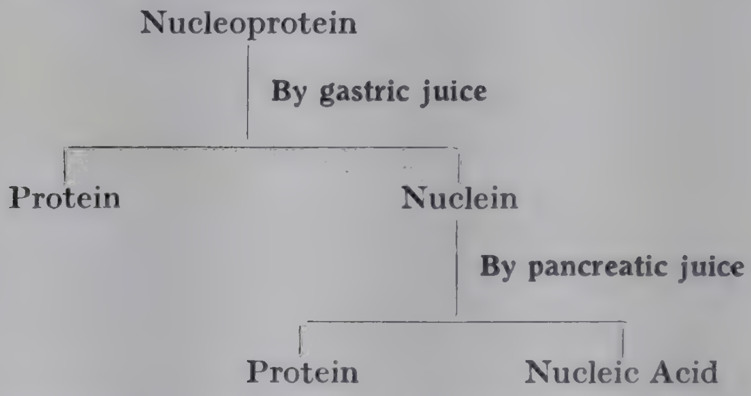
* Vitellin exists in the eggs as a compound with lecithin, *lecithovitellin*, and is therefore sometimes described as a lecithoprotein.

† According to Jorpes, *heparin*, the substance present in tissues which prevents blood clotting, is probably similar in structure to mucoitin sulphuric acid.

The occurrence of mucoproteins with other carbohydrates as prosthetic groups is probable.

(8) **Nucleoproteins** (32, 33). These are compounds of proteins with nucleic acid. They are found in all cell nuclei and the protoplasm of cells, most abundantly in glandular tissues like thymus or pancreas.

Owing to the nucleic acid, they contain both carbohydrate and phosphoric acid, but most characteristic is the inclusion of purine and pyrimidine bases. They are probably best regarded as loose compounds of nucleic acid with varying proportions of protein. The protein moiety may be a histone or a protamine. The protein can usually be removed in two stages by careful hydrolysis with dilute acids or the enzymes of gastric and pancreatic juices respectively. The ill-defined compound obtained as an intermediate product is called **nuclein**. The complete hydrolysis can be represented thus :—



The structure of nucleoproteins, owing to their instability, has been little investigated. Attention has been almost entirely devoted to the nucleic acids, for which see Chap. VIII.

Nucleoproteins of special interest are the **virus proteins**. Several crystalline proteins of exceptionally high molecular weight have been isolated from the juice of plants affected by virus diseases, *e.g.*, tobacco mosaic, tomato bushy stunt; no proteins of such high molecular weight are detectable in the juice of the healthy plants. The protein of tobacco mosaic disease has a molecular weight of about 17,000,000 and an isoelectric point of pH 3·49, and contains about 5% of nucleic acid. Even after several recrystallisations minute amounts of virus proteins induce the virus disease when injected into healthy plants. One millionth of a milligram of tobacco mosaic protein invariably infects a healthy plant. From the infected plant relatively large quantities of the same protein can be isolated. This property, of directing the metabolism of the plant so as to synthesise more of the particular protein injected, is unique in a chemical compound and is suggestive of the reproduction of a living organism rather than the elaboration of a non-living molecule. The evidence at present available points to the purely chemical nature of these giant molecules, although we have no absolute proof that they are inanimate. A homogeneous preparation of a protein of very high molecular weight (47,000,000) has been obtained from the warty tissue of virus-induced rabbit papillomatosis. This protein is probably the causative agent of the disease.

Recent work on the nature of the **chromatin** of chromosomes suggests that it is essentially nucleoprotein composed of thymonucleic acid and protamine.

(9) **Chromoproteins** (4, 10). These are proteins with a coloured prosthetic group. The best known, the **hæmoglobins** (see Chap. XII), contain iron in the prosthetic group. **Cytochrome** (p. 172) might be included in this group. The **hæmocyanins** (p. 119), found in the blood of certain invertebrates, are also chromoproteins with Cu in their prosthetic group. Visual purple, **rhodopsin** (p. 121), can also be regarded as a chromoprotein.

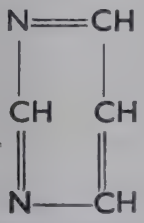
CHAPTER VIII

NUCLEIC ACIDS (4, 32, 33)

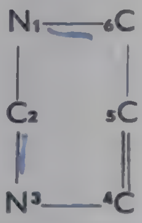
So far as is known, nucleic acids only exist in nature in association with protein as nucleoprotein. They were first discovered in the nuclei of pus cells from which they were separated after digestion of the rest of the protoplasm with gastric juice. Nucleic acids are complex substances which on hydrolysis with acids give phosphoric acid, carbohydrate and organic bases belonging to the classes known as *pyrimidines* and *purines.*

Pyrimidines

The parent substance of this group, pyrimidine, is a six-membered ring composed of 4C and 2N atoms.

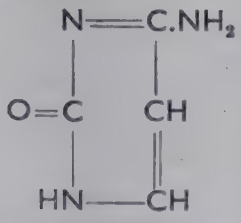


PYRIMIDINE



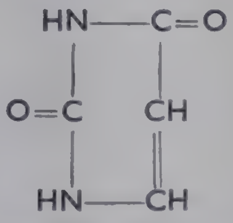
Conventional Numbering

Three derivatives of this compound are found in nucleic acids. They are :—



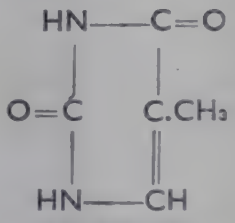
CYTOSINE

(6-Amino-2-oxypyrimidine)



URACIL

(2, 6-Dioxypyrimidine)



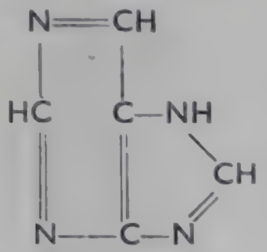
THYMINE

(5-Methyl uracil)

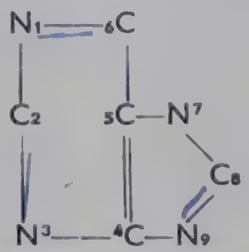
They are obviously closely related. **Uracil** would be formed from **cytosine** on oxidation of the NH_2 and **thymine** is a methyl derivative of uracil. It should be noted that all these three substances can be considered derivatives of urea, *i.e.*, they are cyclic ureides formed from urea and a suitable 3-carbon compound (substituted with CH_3 in the case of thymine). The skeletons of urea will be seen on the left of the formulæ. Pyrimidines can be synthesised from urea.

Purines

These compounds can be considered as products of the condensation of pyrimidines with urea. The parent compound, **purine**, has the following formula :—



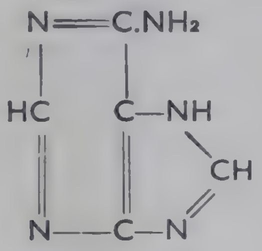
PURINE



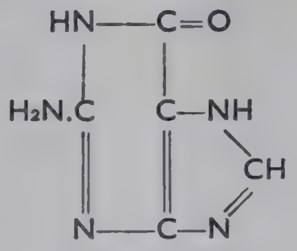
Conventional Numbering

The skeletons N—C—N of urea are separated by the chain of 3 carbon atoms. A derivative of purine (uric acid) has been synthesised from 2 molecules of urea and 1 molecule containing 3 carbon atoms. Note also that the five-membered ring (*glyoxaline* or *iminazole* ring) appears in histidine.

In nucleic acid only two purine derivatives are found, **adenine** and **guanine.**

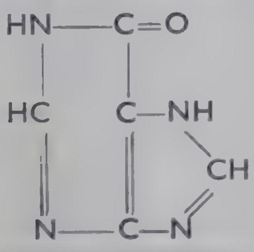


ADENINE
(6-Aminopurine)

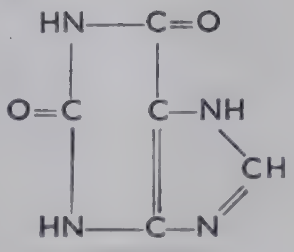


GUANINE
(2-Amino-6-oxypurine)

On oxidation of the amino group, these compounds give **hypoxanthine** and **xanthine** respectively.



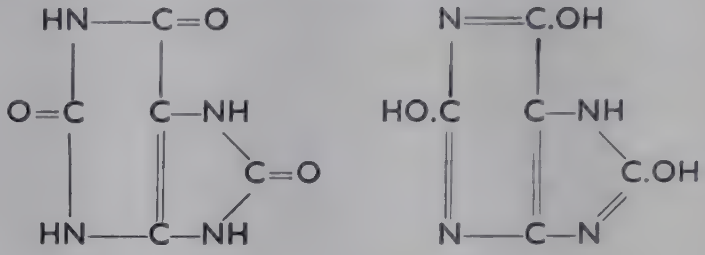
HYPOXANTHINE
(6-Oxypurine)



XANTHINE
(2, 6-Dioxypurine)

Oxidation of hypoxanthine yields xanthine, which, on further oxidation, gives 2, 6, 8-trioxy purine, better known as **uric acid.** 1, 3, 7-trimethyl xanthine, found in tea leaves, is known as **caffeine** and used therapeutically as a stimulant.

Uric Acid. Uric acid is important as an end product of purine metabolism. It exists in both keto (*lactam*) and enol (*lactim*) forms.



Lactam Form Lactim Form

URIC ACID

Both forms are probably present in a solution of uric acid. Its acidic properties are due to the hydroxyl groups of the enol form, but at physiological pH ranges only one OH is sufficiently acidic to form salts.

It is a stronger acid than carbonic, but weaker than acetic. Uric acid is only slightly soluble in water (6·5 mg. per 100 c.c. at 37° C.); its salts are more soluble.

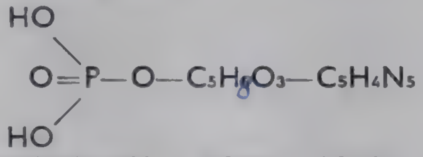
The substance deposited in the joints in gout is said to be a double compound of monosodium urate and uric acid, sometimes called sodium quadri-urate.

NUCLEIC ACIDS

Two types of nucleic acids are known—*ribonucleic acids* containing ribose and *desoxyribonucleic acids* containing desoxyribose. Formerly the ribonucleic acids were thought to be all alike and exclusive to plants, whereas one desoxyribonucleic acid was allotted exclusively to animal tissues—hence the description *plant nucleic acid* and *animal nucleic acid.* But there are certainly more than two nucleic acids, and both a ribonucleic acid and a desoxyribonucleic acid have been obtained from pancreas, rye and yeast. The acids which have been most closely examined are the desoxyribonucleic acid of the thymus gland, *thymonucleic acid*, and the ribonucleic acid of yeast, *yeast nucleic acid.* These acids yield the following products on hydrolysis :—

Thymonucleic Acid	**Yeast Nucleic Acid**
Adenine.	Adenine.
Guanine.	Guanine.
Cytosine.	Cytosine.
Thymine.	Uracil.
d-2-Desoxyribopyranose.	*d*-Ribofuranose.
Phosphoric acid.	Phosphoric acid.

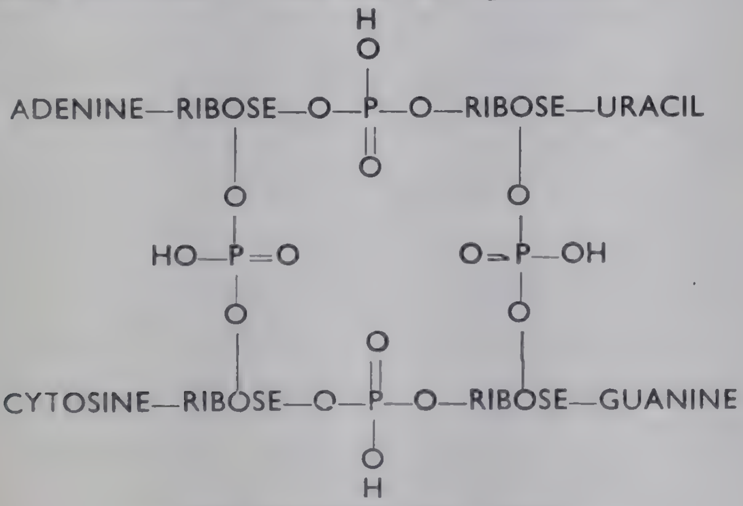
Note the very close similarity between the components. They differ only in respect of one pyrimidine and the carbohydrate, and even here the compounds are very closely related. There is 1 molecule of phosphoric acid and 1 molecule of sugar to every one of base. The nucleic acids are, in fact, almost certainly composed of ~~four~~ three units, called *mononucleotides*, each consisting of phosphoric acid, sugar and base (1 molecule of each). These mononucleotides have been obtained by careful hydrolysis of nucleic acid. The sugar forms the connecting link between the phosphoric acid and base thus :—



$$(HO)_2(O{=})P{-}O{-}C_5H_8O_3{-}C_5H_4N_5$$

Phosphoric Acid Ribose Adenine

Yeast nucleic acid has a molecular weight (about 1,300) consistent with a tetranucleotide. Recent experiments with enzymes which attack organic phosphates suggest that *all* the phosphoric acid groups are doubly esterified with ribose, indicating a cyclic arrangement of the nucleotides rather than an open chain as in the older formulæ. This can be represented :—

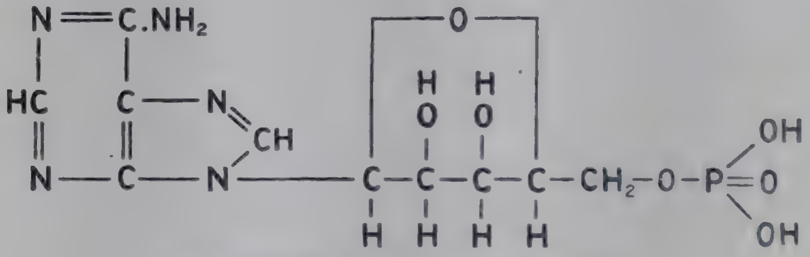

ADENINE—RIBOSE—O—P—O—RIBOSE—URACIL
HO—P=O
O=P—OH
CYTOSINE—RIBOSE—O—P—O—RIBOSE—GUANINE

YEAST NUCLEIC ACID

The relative positions of the bases are arbitrary.

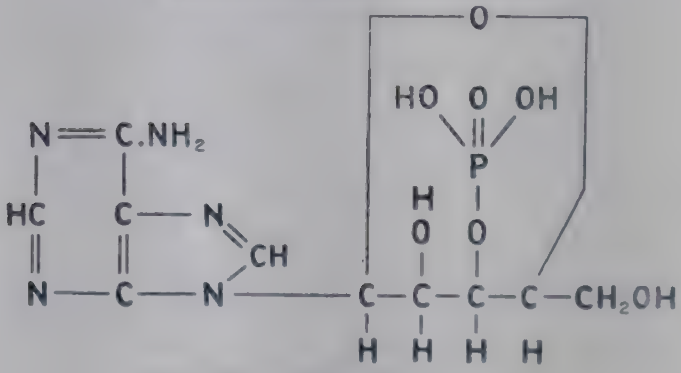
Thymonucleic acid, once thought to resemble yeast nucleic acid in structure, is revealed as far more complex by X-ray analysis and other physical measurements. Its molecular weight lies between 500,000 and 1,000,000, and it appears to consist of a column of about 2,000 mononucleotide units in which the pattern repeats not after four but after at least seventeen mononucleotides. The idea of thymonucleic acid as a tetranucleotide must, therefore, be abandoned.

Mononucleotides. Free mononucleotides and their derivatives are found in the tissues. Two, **adenylic acid** and **inosinic acid,** exist in muscle; in the former adenine is the base, in the latter hypoxanthine. A guanine mononucleotide, **guanylic acid,** is found in yeast, liver, pancreas and spleen. The sugar of these three mononucleotides and of those obtained by hydrolysis of yeast nucleic acid is ribofuranose. The phosphoric acid is attached to carbon 3 of the ribose in the mononucleotides of yeast nucleic acid and guanylic acid, but in muscle adenylic and inosinic acids the union is through carbon atom 5. The place of attachment in the desoxyribopyranose nucleotides of thymonucleic acid is not yet known owing to the difficulty of isolating them intact. The adenine mononucleotide from yeast nucleic acid is known as **yeast adenylic acid.**



MUSCLE ADENYLIC ACID

(9′-Adenine-5-phosphoribofuranoside)



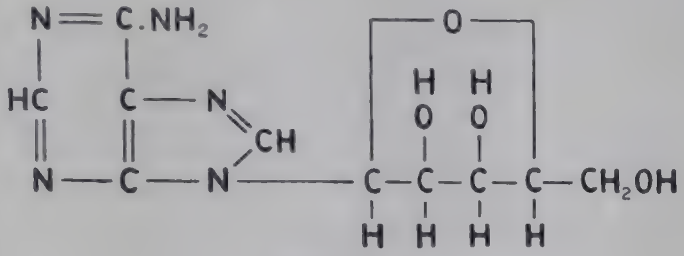
YEAST ADENYLIC ACID

(9′-Adenine-3-phosphoribofuranoside)

Nucleosides. Whereas acid hydrolysis of a mononucleotide yields a sugar phosphate, alkaline hydrolysis splits off the phosphoric acid, leaving the sugar attached to the base; this can be represented :—

$$\mathrlap{\underbrace{\phantom{\text{PHOSPHORIC ACID—SUGAR}}}_{\text{Acid Hydrolysis}}}\text{PHOSPHORIC ACID—}\overbrace{\text{SUGAR—BASE}}^{\text{Alkaline Hydrolysis}}.$$

The compound of sugar and base is called a *nucleoside*. The linkage in these compounds being through carbon 1 of the sugar, they are glycosides, or, more specifically, ribosides. The purines are most probably connected through nitrogen 9 and the pyrimidines probably through nitrogen 3.



ADENOSINE
(9-Adenine Ribofuranoside)

Adenylic acid gives **adenosine,** guanylic acid **guanosine** and inosinic acid **inosine.** The same adenosine will, of course, be obtained from either muscle or yeast adenylic acid. Guanosine and inosine occur in the tissues as such. The nucleoside of xanthine, **xanthosine,** may be formed when guanosine is oxidised in the body. A uric acid riboside has been found in blood and in liver.

The desoxyribosides from thymonucleic acid have been isolated; the sugar is probably attached to the same nitrogen atoms as in the ribosides.

CHAPTER IX

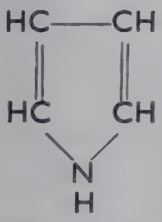
ANIMAL PIGMENTS (4, 9)

This chapter should be omitted at the first reading of the book.

In view of the recent discoveries of the functional importance of many animal pigments, it may be of interest to consider them as a group, although the more important members are discussed in detail elsewhere.

Pyrrole Pigments

A very large number of animal pigments containing pyrrole nuclei are known. Four substituted pyrrole nuclei are joined together through methinyl (= CH—) or methylene ($—CH_2—$) groups to form a chain or a ring. The chain type provides the **bile pigments** and the ring type the **porphyrins**, which have the property of combining with metals and proteins to give pigments active in the carriage of respiratory gases or in oxidation-reduction systems. The more important of these are :—



PYRROLE

Ring Type	*Chain Type*
Hæmoglobins.	Verdohæmochromogen.
Hæmochromogens.	Biliverdin.
Cytochromes.	Bilirubin.
Hæmatin.	Urobilin.
Hæm.	
Protoporphyrin.	
Coproporphyrin.	
Uroporphyrin.	

These compounds and the part they play are described on pp. 169 to 179. The commonest plant pigments, the **chlorophylls** (p. 173), are also porphyrin derivatives. Porphyrins are not restricted to the higher animals, and are found in several lower organisms.

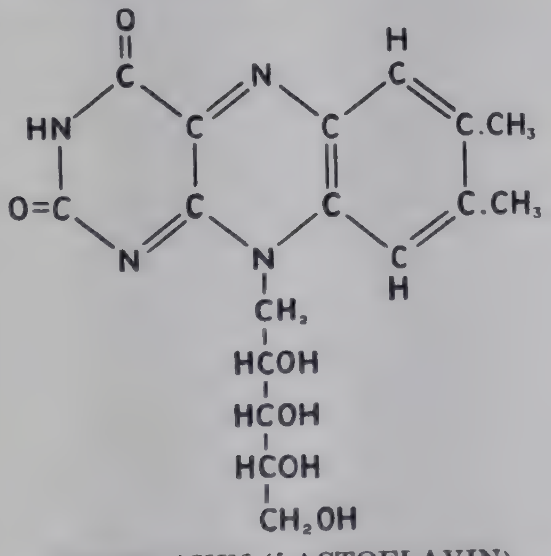
PORPHYRIN DERIVATIVES IN LOWER ORGANISMS

Pigment	Colour	Metal	Organism
Hæmocyanin . .	Blue	Cu	Molluscs. Crustaceans.
Helicorubrin * . .	Red	Fe	Molluscs. Crayfish.
Actiniohæmatin * . .	Red	Fe	Sea anemones.
Chlorocruorin . .	Green	Fe	Polychæte worms.
Pinnaglobulin . .	Brown	Mn	Pinna squamosa.
?	Brown	V	Ascidians.

* The prosthetic group is probably hæm.

Flavins (Lyochromes)

Almost all cells contain water-soluble nitrogenous yellow fluorescent pigments, which are closely related to, if not identical with riboflavin or vitamin B_2 (p. 361). These pigments are called *flavins* (lactoflavin, ovoflavin, cytoflavin) or *lyochromes*, and are associated naturally with phosphoric acid and protein, forming oxidation-reduction systems (p. 142). Riboflavin has been synthesised and has the formula :—



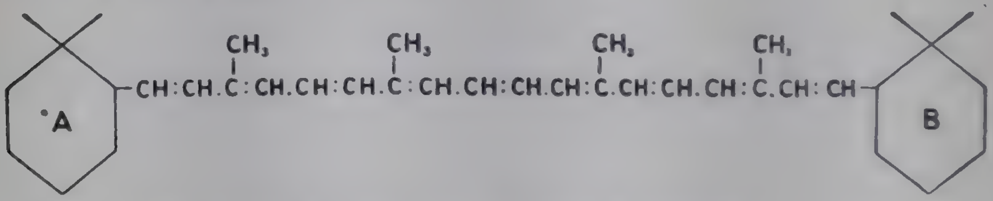
RIBOFLAVIN (LACTOFLAVIN)

(The sugar alcohol is that of *d*-Ribose. Note the pyrimidine ring on the left.)

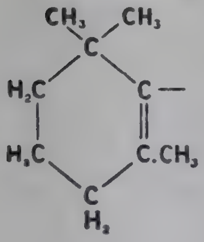
These pigments are only present in minute amounts. 180 mg. of ovoflavin are obtained from the whites of 10,000 eggs, and 1 g. lactoflavin from 5,400 litres of whey.

Lipochromes (Carotenoids)

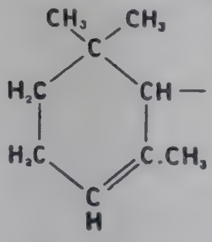
The fat extracted from any tissue is always more or less yellow. This is due to the presence of fat-soluble pigments called *lipochromes* or *carotenoids*. Those which have been identified in animal tissues are orange-coloured unsaturated hydrocarbons or alcohols containing 40 carbon atoms, *e.g.*, **carotenes**, $C_{40}H_{56}$ (three forms, α, β and γ), and **xanthophylls**. They are probably ultimately derived from plant sources and are most easily obtained from carrots (25 g. carotene from 1,000 kg.) and plant leaves respectively. Many closely related pigments have been identified in plants, *e.g.*, **lycopene** in tomatoes. Carotenoids can be represented by the general formula :—


CH_3
CH_3
CH_3
CH_3
CH:CH.C:CH.CH:CH.C:CH.CH:CH.CH:C.CH:CH.CH:C.CH:CH
A
B

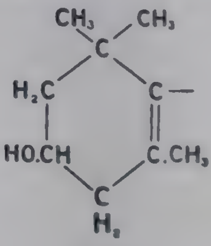
in which rings A and B are one of the following :—


CH_3
CH_3
C
H_2C
C—
H_2C
$C.CH_3$
C
H_2

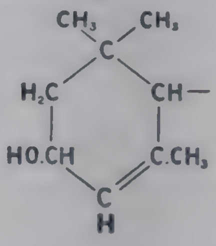
I


CH_3
CH_3
C
H_2C
CH—
H_2C
$C.CH_3$
C
H

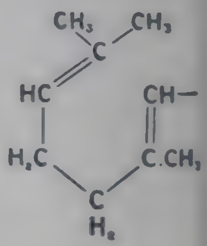
II


CH_3
CH_3
C
H_2C
C—
HO.CH
$C.CH_3$
C
H_2

III


CH_3
CH_3
C
H_2C
CH—
HO.CH
$C.CH_3$
C
H

IV


CH_3
CH_3
C
HC
CH—
H_2C
$C.CH_3$
C
H_2

V

		Ring A	*Ring B*
α-Carotene		I	II
β-Carotene		I	I
γ-Carotene		I	V
Kryptoxanthin	(Xanthophylls)	I	III
Lutein		III	IV
Zeaxanthin		III	III
Lycopene		V	V

The carotenes can be broken down to vitamin A in the animal liver (p. 350). Egg-yolk contains lutein and zeaxanthin, and corpus luteum carotene. Carotenes and xanthophylls are present in body fat, milk fat, serum and nervous tissue. Lycopene has been identified in human fat. The pigments are only present in small amounts. The pigments of canary's feathers and goldfish scales are also carotenoid in nature.

Melanins

Black pigments, melanins, are widely distributed in animals. In man they are found in the skin of negroes or in whites in sun-tan, moles, melanotic tumours, and the pigmented tissues in ochronosis, as well as in the hair and choroid of the eye. On the basis of Raper's study of the oxidation of tyrosine to black pigments (p. 281) it is inferred that many melanins are formed from this amino-acid or related substances, *e.g.*, catechol derivatives like adrenaline. The extensive brown pigmentation of the skin in disorders of the suprarenal glands (*e.g.*, Addison's disease) is of interest in this respect.

Other Pigments

The photochemically active dye in the rods of the retina, **Visual Purple** or **Rhodopsin**, can be regarded as a chromoprotein. When it is bleached by light, it breaks down, giving amongst other products vitamin A (p. 348). A blue protein found in mammalian erythrocytes, *hæmocuprein*, contains about 0·34 per cent. of copper, corresponding to two atoms per molecule. Polyphenoloxidases also contain copper. The nature of the coloured prosthetic groups of these proteins has not been established.

For the pigments of urine see Chapter XXXII.

CHAPTER X

ENZYMES (1, 9, 62)

ENZYMES may be defined as organic catalysts produced in the living organism. To distinguish them from other catalysts which may be found in the body, they should be further specified as soluble and colloidal. Although they are produced in the living cell, they are not dependent upon the " vital " processes of the cell and will work outside the cell.

At one time enzymes were divided into two classes, the " *unorganised* " and " *organised ferments*," according as they worked independently (*e.g.*, in gastric juice) or associated with living cells (*e.g.*, in fermentation of sugar by yeast). The action of " organised ferments " was thought to be due to " vital " activity, a view which was encouraged by the " rediscovery " of bacteria by Pasteur and Koch in the middle of the last century. These workers established the connection between bacteria and abnormal (*e.g.*, lactic acid) fermentations in the brewing vat and in action the putrefaction of meat. The " vital " conception of enzyme was only finally discredited in 1897 by Buchner, who separated active enzymes from the living cells of yeast and (later) of bacteria.

The features of enzyme action which demarcated them so clearly was the extraordinary ease with which they were destroyed by slight alteration in acidity or by a rise of temperature ; since these are the conditions which kill cells it is not surprising that the " vital " theory of enzyme action persisted during forty years of frequent but vain attempts to isolate enzymes from the cells.

We now know that enzymes promote and control not only the conversion of the complex carbohydrates, fats and proteins of our food into simple substances which the intestines can absorb, but also the numerous reactions by which these simple substances are utilised in the body for building up new tissues or producing energy. The enzymes are not, however, components in the reactions concerned, nor are they themselves broken down or ultimately changed in the process. They are as potent at the end of a reaction as at the beginning, and very small amounts can effect the conversion of large amounts of material. They are, in fact, true catalysts.

The delicacy and efficiency of these tools elaborated by the cell can best be seen by comparing the conditions under which the same reactions can be performed in the laboratory without their aid. (Many reactions catalysed by enzymes cannot be achieved in the laboratory.) Hydrolysis of a protein by purely chemical means requires the action of strong acid at 100° C. for at least a day; the same change is effected in the alimentary canal at 37° C. and near the neutral point in a few hours. Synthesis of a protein has never been achieved in the laboratory without the aid of enzymes. The acid used for promoting hydrolysis in the laboratory will bring about the hydrolysis of a large number of varied substances such as fats, carbohydrates and esters, but each enzyme will only attack a special class of substances or even a single substance. This specificity is of great importance in the animal, for it enables all reactions to be precisely controlled. For example, fat can be hydrolysed in a cell without affecting any other cell constituents such as the proteins.

Many enzymes have been highly purified and shown to be intensely active. The most active obtained so far is probably the peroxidase from horse-radish, which Willstätter and Pollinger purified so that 1 g. of purified preparation had the activity of 20,000 g. of original material. This preparation activated 1,000 times its own weight of H_2O_2 *per second* at 20° C. Even enzymes which have not been purified to such an extent can effect the change of many times their own weight of substance in a second. It is probable that in many cases the total number of molecules of an enzyme in a cell is very small.

The forty years of active study of enzymes stimulated by Buchner's revolutionary experiments has revealed an imposing array of different enzymes, so that it is difficult now to think of a biochemical reaction for which an enzyme cannot be quoted. This in its turn has given rise to a special terminology.

Enzyme Terminology

First let us amplify our former definition. *Enzymes are soluble, colloidal organic catalysts formed by living cells, but independent of the presence of the latter for their action. They have specific powers of catalysis and are destroyed by moist heat at* 100° *C.* Enzymes which are used in the cells which make them are called **intracellular** enzymes and correspond to the old "organised ferments." Other cells produce enzymes and secrete them to other parts of the body (*e.g.*, digestive juices); these enzymes are described as **extracellular** and correspond to the old " unorganised ferments."

If an extracellular enzyme is secreted ready for action, it is called a **zymase** secretion, *e.g.*, the ptyalin of saliva is secreted complete with activator (p. 131) and at the right pH for action. Other enzymes are secreted in inactive form and subsequently activated by an agent secreted by other cells. This is known as a **zymogen** secretion, and is probably a protective mechanism to prevent digestion of cell walls and ducts, since it is most frequently found with protein-splitting enzymes. Examples of zymogen secretion are :—

Trypsinogen (in pancreatic juice) activated by enterokinase (in intestinal mucosa) to give active trypsin (p. 260).

Prothrombase (in blood) activated by thrombokinase (in tissues) to give active thrombase (p. 151).

The substance or substances upon which an enzyme acts is called the **substrate**; *e.g.*, sugar is the substrate upon which the enzyme of yeast acts to form alcohol.

Apart from a few enzymes, such as ptyalin, pepsin, trypsin and erepsin, whose names have been established by long usage, enzymes are usually named by adding the suffix **-ase** to the main part of the name of the substrate upon which they act. For example :—

Amylases act upon Starch (Amylum).
Carbohydrases act upon Carbohydrates.
Lactase acts upon Lactose.
Lipases act upon certain Lipides.
Maltase acts upon Maltose.
Proteases act upon Proteins.

But there are many substances which may be acted upon by several enzymes in different ways. Thus a dipeptide could be attacked by three enzymes thus :—

A dipeptidase could hydrolyse it to amino-acids, another enzyme could remove the free amino group, and yet another the free carboxyl group. Obviously confusion would result if all these three enzymes were labelled dipeptidases. And so some enzymes are labelled by their *functions* rather than their substrate; the substrate name is usually left for a hydrolysing enzyme. In the example quoted, our three enzymes would be a dipeptidase, a deaminase and a decarboxylase. Other enzymes named by their function are aldehydases, dehydrogenases, hydrolases, oxidases and reductases. Since many enzymes with the same function may act upon one substance, it is good policy to specify the enzyme by its source, *e.g.*, pancreatic amylase, bone phosphatase, liver esterase.

Enzymes acting upon the three main groups of foodstuffs are often conveniently described by the adjectives *amylolytic*, *lipolytic* and *proteolytic*.

Properties of Enzymes

Enzymes cannot be detected or estimated in the strict chemical sense, since they have no distinctive chemical properties except great instability. There is no known reaction in which enzymes play a part other than as catalysts. Their presence can only be revealed by what they can do catalytically. Their destruction by moist heat at 100° C. provides us with a criterion for distinguishing them from other catalysts. If by adding a neutral solution to some starch at 37° C. we obtain sugar, we can conclude that a catalyst is present; if the neutral solution after boiling fails to produce sugar under similar conditions, we may then conclude that the catalyst is an enzyme and not an inorganic catalyst. This is why in all tests and experiments on enzymes it is essential to carry out a control test with the boiled enzyme solution alongside the test.

Preparation and Isolation. When the great ease with which enzymes were destroyed was realised, methods of isolating enzymes from tissues were soon devised. For purposes of demonstration of their action, tissues can be extracted with saline or glycerol, but such extracts do not keep for any length of time. Stable preparations are made by careful drying of the tissue at low temperature or with some non-injurious water-removing agent such as alcohol or acetone; the product is then ground to a fine powder which keeps well; it is extracted with a suitable solvent when required for use. More active preparations are made by various processes of purification involving dialysis to remove inorganic impurities, adsorption on suitable material with subsequent elution, or precipitation with suitable reagents, all the manipulations being performed at a low temperature and at a reaction not far removed from the neutral point. So much inactive material can be removed in this way that it is certain that enzymes are only present in tissues in extremely small amounts. They must be substances of tremendous potency.

Upon such lines highly active preparations have been obtained; several enzymes, including urease, pepsinogen, pepsin, trypsinogen, trypsin, chymotrypsinogen, chymotrypsin, catalase, carboxy peptidase, tyrosinase, yellow enzyme, carbonic anhydrase, animal phosphorylase, and ascorbic acid oxidase, have been obtained as crystalline proteins which are regarded as the pure enzymes. It is most probable that all enzymes are proteins and that their functions are dependent upon the presence of a suitable prosthetic group (see Co-enzymes, p. 129). Catalase and peroxidase, for example, consist of an iron-containing compound similar to hæmatin associated with a specific protein.

The properties of highly purified enzymes may differ considerably from those in the natural state. Many enzymes, especially proteases, become more specific on purification, due to separation of other enzymes admixed with the original material.

The Conditions of Action of Enzymes

The rate at which an enzyme acts upon its substrate is influenced by a number of factors.

(1) **Contact between Enzyme and Substrate.** This is a matter of great importance, especially in digestion. Rapid digestion is largely dependent on good admixture of food and enzymes, since all the food is not in solution. Fats are an outstanding example. Their digestion is greatly facilitated by fine emulsification of the fat providing a greater area of contact for the lipases. There is probably a physical or chemical union of enzyme and substrate; when the substrate is insoluble the enzyme is usually adsorbed on it, often specifically. Since the substrates upon which enzymes act are often colloidal, it is not to be expected that enzyme reactions follow simple laws *in vitro*, for even the physical conditions in a system change during the reaction, *e.g.*, the hydrolysis of a colloid to crystalloids. Conversely, the synthesis of a colloid from crystalloids might be checked by adsorption of the catalyst on the synthesised molecules. *In vivo*, different conditions prevail and reactants can be more effectively removed from the sphere of action, so that enzyme action in the cell is far more efficient.

(2) **Concentration of Enzyme.** The amount of enzyme does not determine the final equilibrium, but only the time taken to reach this equilibrium. Given sufficient time, a minute amount

of enzyme will produce the same change as a large amount, provided no inhibiting factors intervene. Except when the enzyme is present in excess, the rate of action of an enzyme is sufficiently proportional to the amount of enzyme present to allow the relationship to be used as the basis of methods for assaying the potency of a preparation. Actually the relationship only holds accurately when substrate and reactants form true solutions as in the hydrolysis of a disaccharide.

(3) **Concentration of Substrate.** The rate is also influenced by the amount of substrate. For a given amount of enzyme the reaction rate increases with increasing concentration of substrate until a certain level is reached, when it becomes constant. A very high concentration of substrate may inhibit the action of an enzyme. These effects can probably be ascribed to the conditions of union of enzyme and substrate.

(4) **Temperature.** The velocity of an ordinary chemical reaction is roughly doubled or trebled by a 10° C. rise in temperature. This rule does not hold with enzymes. A rise in temperature accelerates an enzyme reaction, but at the same time causes increasing inactivation of the enzyme. At a certain temperature, therefore, these two effects will balance so as to give a point of maximum activity. This point is known as the **optimum temperature,** and for most animal enzymes is in the region of 37° C. Exposure of such enzymes to temperatures of 60° C. usually inactivates them. Boiling at 100° C. almost invariably * destroys them. Some plant enzymes have a greater heat stability and higher optimal temperatures.

It must be realised that the optimum temperature is not a definite point and will depend upon the method of determination. Thus, if only a relatively small amount of substrate is used, the action will be over quickly and there will be less time for inactivation of the enzyme. Consequently a higher optimum temperature would be recorded than if a large amount of substrate requiring a long time for conversion were used.

The optimal temperatures of animal enzymes lie in the region of 40° C., that of plant urease 60° C.

If the temperature is lowered, the rate of an enzyme reaction is diminished. At a temperature of 0° C. most enzymes are practically inactive. This fact is of importance in the experimental manipulation of tissues to prevent action of intracellular enzymes, *e.g.*, in the preparation of insulin.

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* Under certain conditions acid solutions of purified trypsin are remarkably stable to heat.

(5) **Hydrogen Ion Concentration.** Enzymes are very sensitive to the reaction of their environment. A small change in pH may effectively inhibit their action. Pepsin only works in an acid medium and is inactivated by making it alkaline. Trypsin, on the other hand, which normally works in alkaline solution, will not digest a protein in acid solution. If a curve be drawn comparing the activity of an enzyme on a given substrate with the pH of the mixture, it will reveal a maximum activity at a definite pH, the activity on each side of this value being less. This value is known as the **optimum pH.** Some typical values are given in the table. These values, like optimal temperatures, are not definite and are affected by the presence of interfering substances. They may be considerably changed by purification. The optimum pH determined *in vitro* may not be the same as that *in vivo.* In some cases different values are obtained with different buffers.

Enzyme Class	Enzyme or Source	Optimum pH
Protease . .	Pepsin	1·5
	Trypsin	8·0
	Erepsin	7·7
Amylase . .	Ptyalin	6·9
	Pancreatic	7·0
	Malt	5·2
Phosphatase .	Bone	9·5
	Plant	3·4–6·0
Lipase . .	Pancreatic	8·0
	Gastric	6·0

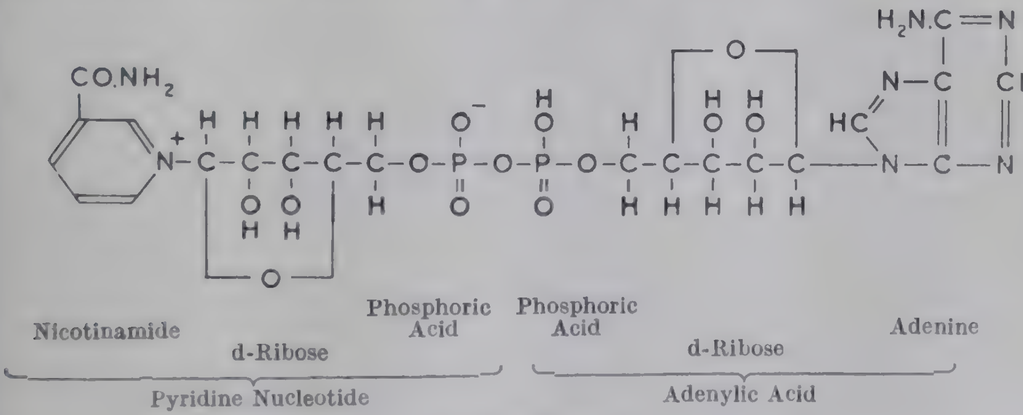
The values in the table show that the optimum pH is unrelated to the function of the enzyme, so that the same chemical reaction could be carried out in the body in two localities of different pH. The biological significance of optimum pH lies in the possibility of control of reactions by fine adjustment of pH.

(6) **Co-enzymes and Activators.** Some enzymes only work efficiently if some other specific substance is present. This other substance, which may be an inorganic ion or an organic compound,

has been described as an activator or a co-enzyme. In the past there was a tendency to regard these terms as synonymous, and it is only recently that they have become clearly distinguished.

Co-enzymes (29, 77, 81). In the modern sense of the word a co-enzyme is an organic substance specifically associated with the enzyme. Recent work suggests that an enzyme is a conjugated protein in which the co-enzyme forms the prosthetic group. In some enzymes the prosthetic group is labile, and therefore easily separated from the protein component.* Other enzymes have the prosthetic group more firmly attached, *e.g.*, peroxidase, xanthine oxidase, so that they appear not to require a co-enzyme. No prosthetic group, however, has been separated from the proteolytic enzymes. Several co-enzymes have been isolated and shown to play the part of " carriers," for example, of hydrogen in dehydrogenases (*e.g.*, co-carboxylase, p. 130) or of phosphoric acid (adenylic acid, p. 239). The co-enzyme accepts, say, hydrogen from the substrate, the reduced co-enzyme subsequently gives up this hydrogen to a suitable hydrogen acceptor and is thereby regenerated. The function of the protein part of the enzyme is to establish contact between the co-enzyme and the substrate.

Several co-enzymes of enzymes concerned with oxidation and reduction contain phosphoric acid. Most of these could be described as nucleotides in which the basic groups are members of the B group of vitamins (aneurin, riboflavin, nicotinamide and pyridoxin, pp. 359–363). The formulæ of three of these co-enzymes are set out below, another is given on p. 142.



CO-ENZYME I

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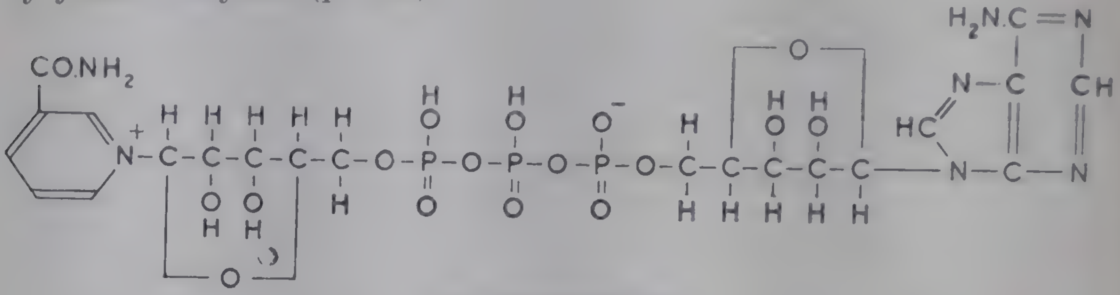
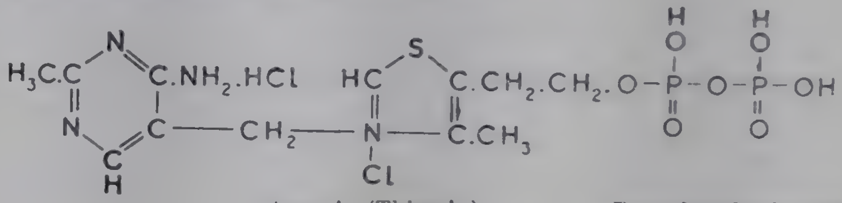
* The protein component has been called the *apo-enzyme* and the complete enzyme (apo-enzyme + co-enzyme) the *holo-enzyme.*

Co-enzyme I (*Cozymase I, Diphosphopyridine nucleotide*) has been shown to be a co-enzyme for at least thirty-five different reactions. It accepts hydrogen at the double bond attached to the pyridine nitrogen atom forming **dihydroco-enzyme I.** This is oxidised back to co-enzyme I by yellow enzymes (p. 142).



CO-ENZYME II

Co-enzyme II (*Cozymase II, Triphosphopyridine nucleotide*) accepts hydrogen forming **dihydroco-enzyme II** in the same way as co-enzyme I and acts as a co-enzyme in many reactions. It differs from co-enzyme I in having an additional phosphoric acid.



Aneurin (Thiamin) Pyrophosphoric Acid

COCARBOXYLASE

Cocarboxylase (*Aneurin phosphate*, p. 359) is a co-enzyme to several enzymes. It accepts two atoms of hydrogen at the double bond attached to the nitrogen atom in the thiazole ring.

Examples of some co-enzymes and their holo-enzymes are given in the following table.

Co-enzyme	Holo-enzyme	Action
Adenylic Acid.	Animal Phosphorylase.	Cori ester ⟶ glycogen.
Cocarboxylase.	Carboxylase.	Pyruvic acid ⟶ acetaldehyde.
Co-enzyme I.	Many Dehydrogenases.	Oxidation of alcohol, lactic acid, glucose, etc.
Co-enzyme II.	Many Dehydrogenases.	Oxidation of glucose, glutamic acid, citric acid, hexosephosphate, etc.
Cytoflavin	Old Yellow Enzyme.	Oxidation of hexosephosphate.
Isoalloxazine adenine. dinucleotide.	Xanthine Oxidase.	Oxidation of xanthine, hypoxanthine, aldehydes, etc.
Isoalloxazine adenine dinucleotide.	d-Amino-acid Oxidase.	Oxidation of d-amino-acids.
Glutathione.	Glyoxalase.	Methyl glyoxal ⟶ lactic acid.

Activators. These can be described as substances which specifically increase the activity of a complete enzyme (protein + co-enzyme). In the absence of the activator the enzyme may be inactive or sluggish. Activators are usually inorganic ions, although organic activators are known. Typical activating ions are Cl′ for ptyalin, Mg·· for plasma phosphatase, and Ca·· for thrombase. The function of an activator is not always clear. In some instances it is a prevention of the inactivation of the enzyme by some inhibiting agent, in others the activator can be shown to play some part in the reaction, whilst in others no satisfactory explanation has been put forward.

Activators which are enzymes are called **kinases**, *e.g.*, enterokinase, thrombokinase. The former probably acts by prevention of inactivation (p. 260).

(7) **Inhibiting Agents.** Just as enzymes may be assisted by the presence of certain substances, so they may be inhibited or inactivated by others. Salts of mercury, silver and gold are especially potent in this respect. Many enzymes are inhibited by other heavy metal salts or fluorides ; oxidases are usually inhibited by cyanides. Certain preservatives, such as chloroform, glycerol and thymol, whilst innocuous to most enzymes, inhibit some. Toluene appears to be without action on enzymes, and is therefore one of the best preservatives for enzyme solutions. Highly reactive substances, such as aldehydes (*e.g.*, formaldehyde), destroy enzymes.

In many instances the inhibition of an enzyme can be regarded as the result of chemical combination between the enzyme and the inhibitor. An inactivated enzyme can often be reactivated by removing the inhibiting substance (*e.g.*, heavy metals by H_2S).

(8) **Anti-enzymes.** If certain enzymes are repeatedly injected into an animal there is produced in the animal's serum a substance which prevents the normal action of the enzyme injected. This substance is called an **anti-enzyme**. Amongst those known are *anti-pepsin*, *anti-rennin*, *anti-trypsin* and *anti-urease*. It is supposed by some that the reason why the alimentary canal is not digested by its own secretions is that the mucous membrane contains suitable anti-enzymes. Several anti-enzymes have been found (ready formed) in various tissues, *e.g.*, anti-glyoxalase in pancreatic juice. The exact nature of these anti-enzymes is unknown.

Enzymic Synthesis

Some enzymes of the body cause the hydrolysis of a substance, others its synthesis. It is probable that both processes are catalysed by the same enzyme. There is considerable experimental evidence that isolated enzymes can catalyse reactions from either side up to the equilibrium point of the particular reaction. Examples of proved reversible reactions are :—

Enzyme	*Reaction*
Lipase . . .	Triolein ⇌ Oleic Acid + Glycerol.
Maltase . .	Maltose ⇌ Glucose.*
Pepsin . . .	Protein ⇌ Proteose.
Phosphatase .	Glycerophosphate ⇌ Glycerol + Phosphoric Acid.
Trypsin . .	Salmine ⇌ Amino-acids.
Urease . .	Urea ⇌ Ammonium Carbonate.

The yields in the protein syntheses are poor. The reversible action of phosphatase provides a very convenient class experiment. The direction in which an enzyme works is determined by the nature and concentration of the substrate and also the pH. Some enzymes have different optimal pH for hydrolysis and synthesis thus :—

	Hydrolysis	Synthesis
Pepsin . .	1·2	4·0
Trypsin . .	8·0	5·7
Urease . .	7·0	8·7

Specificity

A feature which distinguishes enzymes from inorganic catalysts is their extraordinary specificity. Some are outstanding in only attacking one substance, even when crude preparations of the enzyme are used. As examples arginase, catalase and urease may be mentioned ; these enzymes only attack arginine, hydrogen peroxide and urea respectively. Other enzymes are specific for particular groupings, thus there are esterases, phosphatases, glucosidases, peptidases, deaminases, decarboxylases and sulphatases attacking substrates indicated by their names. But these enzymes do not always attack all compounds containing the particular group concerned. A given phosphatase will not attack all phosphoric esters, a given glucosidase will not hydrolyse all glucosides or even closely similar glucosides, *e.g.*, α- and β-methylglucosides. The α-glucosidase, maltase, will not attack the β-galactoside, lactose, nor will the β-galactosidase, lactase, attack the α-glucoside, maltose. Maltase will not even attack the α-glucoside, sucrose. Similar specificity is shown by the peptidases. Dipeptidase only hydrolyses dipeptides. Polypeptidase hydrolyses simple peptides such as tripeptides, but not dipeptides. Mammalian phosphatases usually attack only monophosphoric esters and not diphosphoric esters.

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* This reaction is more accurately expressed

Maltose → Glucose → Isomaltose.

The synthetic product is not maltose, but an isomer, *isomaltose*.

Another type of specificity frequently encountered is the different behaviour with *d*- and *l*-forms of the same substance. Dipeptidase will attack glycyl-*l*-leucine (the natural form), but not glycyl-*d*-leucine ; liver esterase will attack esters of *d*- but not of *l*-mandelic acid. The less specific enzymes are the proteases, lipases and esterases. It may be that these relatively non-specific enzymes are really mixtures of several specific enzymes ; thus the classical trypsin of pancreatic juice is a mixture of a true protease, polypeptidase and dipeptidase. Purified trypsin does not hydrolyse polypeptides or dipeptides.

The probable reason for the specificity of an enzyme is that its action depends upon close contact being established between enzyme and substrate. Some part of the enzyme must fit structurally with the part of the substrate which is attacked. The specificity is due to the protein component of the enzyme. Even the least specific enzymes only attack one kind of linkage, so that attack by the same enzyme may almost be accepted as proof of some common structural feature in two substances, *provided* that the enzyme can be regarded as a single substance.

Autolysis

If tissues are kept under sterile conditions after death, the intracellular enzymes gradually digest the tissues, softening or even liquefying them. This *post-mortem* self-digestion of tissues is called *autolysis*. Glandular tissues autolyse more readily than muscle. This is one reason why the viscera are removed from a carcase intended for consumption as food. The meat is hung to allow autolysis to proceed so as to improve the flavour and digestibility of the meat. It is customary to permit considerable autolysis in game and venison. The intracellular protease present in all mammalian tissues is called **kathepsin.**

Atrophy is essentially a process of autolysis which takes place in the living animal. Autolysis occurs in the living animal in pathological conditions, *e.g.*, wasting in starvation and fevers, yellow atrophy of the liver and liquefaction of pus.

CHAPTER XI

OXIDATION AND REDUCTION (1, 4, 29)

It is easy to talk of a substance being oxidised in the body but when we come to think of how this occurs we realise that the process is by no means simple. There is no doubt that oxidation does occur in the body, oxygen is consumed and carbon dioxide produced, and that the process which is summarised by

$$\text{Food} + O_2 \rightarrow CO_2 + H_2O$$

provides most of the energy required for the functioning of the body. Isolated tissues, or even small slices of tissue, respire by using oxygen and producing carbon dioxide. When we study intermediary metabolism we see that the oxidation of a foodstuff involves many stages. Now in working out these intermediate stages we frequently have to postulate the oxidation of stable substances, which would certainly not be oxidised *in vitro* under the influence of the air or even pure oxygen ; other oxidations do not involve utilisation of oxygen at all, but removal of hydrogen ; with metals an electron is lost and neither oxygen nor hydrogen is involved (*e.g.*, ferrous to ferric).

The burning of carbon monoxide in air is an example of the complexity of an apparently simple oxidation. The reaction is usually recorded $2CO + O_2 = 2CO_2$. Actually it has been proved by isolation of the intermediate products that the oxidation proceeds by the following stages :—

$$\begin{array}{lll} & 2CO + 2H_2O & = 2HCOOH \\ & 2HCOOH & = 2CO_2 + 2H_2 \\ & 2H_2 + 2O_2 & = 2H_2O_2 \\ & 2H_2O_2 & = 2H_2O + O_2 \\ \hline \text{Summated} & 2CO + O_2 & = 2CO_2 \end{array}$$

The evolution of hydrogen in the second stage has actually been demonstrated at 37° C. using a suitable catalyst, so that under certain conditions the oxidation of CO to CO_2 can proceed *in the absence of free oxygen.*

Let us be clear as to the meaning of oxidation. We have three types :—

(1) Addition of oxygen to a molecule,
e.g., $CH_3CHO \rightarrow CH_3COOH$.
(2) Removal of hydrogen from a molecule,
e.g., $CH_3CH_2OH \rightarrow CH_3CHO$.
(3) Loss of an electron, *e.g.*, $Fe^{++} \rightarrow Fe^{+++} + e$.

The first two, applying to organic reactions, are of greater biochemical interest. Now it is important to remember that *every oxidation involves a corresponding reduction of some other substance*, and that in all reactions in the body *water is present.*

Aerobic Oxidation

In oxidation by molecular oxygen the oxygen must be activated. Hæmoglobin is not an oxygen activator ; it is only a transporter of oxygen. The activation is effected indirectly by oxidising enzyme systems called *aerobic oxidases.* While this system is most easily demonstrated in plants, there is evidence that it is applicable to animal cells. The intermediate formation of a peroxide is involved. A substance which takes up oxygen readily (*oxygen acceptor*) combines with oxygen to form a **peroxide** which is a powerful oxidising agent and the peroxide acts as *oxygen donor* to another acceptor. The oxidation of benzaldehyde to benzoic acid could be represented :—

$$\underset{\text{Benzaldehyde}}{C_6H_5CHO} + O_2 \rightarrow \underset{\text{Benzoyl Peroxide}}{C_6H_5CO.O.OH}$$

$$C_6H_5CO.O.OH + C_6H_5CHO \rightarrow 2C_6H_5COOH.$$

If we make indigo the oxygen acceptor in the second reaction instead of benzaldehyde we should have

$$C_6H_5CO.O.OH + \text{Indigo} \rightarrow \text{Oxidised Indigo} + C_6H_5COOH.$$

Or in general terms $A + O_2 \rightarrow AO_2$ . . . . (1)

$AO_2 + B \rightarrow BO + AO$ . . . . . . (2)

where A is a substance which can react with oxygen to give a peroxide and B is a substance which is stable to oxygen, but oxidised by a peroxide.

Reaction (2) is catalysed by an enzyme **peroxidase.** The system A + peroxidase is called an **aerobic oxidase.** A is called an **autoxidisable substance.** (The term oxygenase is better avoided, since it suggests an enzyme.)

Reactions of this type can be easily demonstrated with horse-radish juice which contains a strong peroxidase. Guaiaconic acid (from guaiacum resin) on oxidation gives blue compounds. Guaiaconic acid with either horse-radish juice or hydrogen peroxide gives no blue colour, but with a mixture of the two it does. This is a reaction of type (2), although in this case AO_2 (hydrogen peroxide) is not formed by oxidation of an autoxidisable substance, but added preformed.

$$\underset{(AO_2)}{H_2O_2} + \underset{(B)}{\text{Guaiaconic Acid}} \longrightarrow \underset{(BO)}{\text{Blue compound}} + \underset{(AO)}{H_2O}$$

It does not take place with boiled horse-radish juice. In the browning of a freshly cut potato or apple we can see the complete reaction, for here we have an autoxidisable substance (A) as well as a peroxidase. B in the potato is probably tyrosine (or compounds related to it), which oxidises to a brown compound. If we add guaiaconic acid to potato shavings it is slowly turned blue. The autoxidisable substance in potato does not take up oxygen very rapidly, so AO_2 is formed slowly. If we, in effect, increase AO_2 by adding H_2O_2, guaiaconic acid is much more rapidly oxidised.

In the animal body there are oxidases such as tyrosinase and indophenol oxidase (so called because it forms a blue indophenol derivative from dimethyl-*p*-phenylenediamine and naphthol). It is not certain whether an organic peroxide is formed from an autoxidisable substance, or H_2O_2 (produced in other reactions) forms the peroxide. There is little doubt that oxidase reactions of this general type do take place. There is an extraordinary similarity between oxidations *in vivo* and oxidations *in vitro* with H_2O_2. Certain inorganic substances behave like peroxidases, notably traces of ferrous salts, and accelerate oxidation with hydrogen peroxide. Dakin quotes the following examples of reactions, known to occur *in vivo*, brought about by hydrogen peroxide and ferrous sulphate (Fenton's Reagent) *in vitro* :—

*β-Oxidation of Fatty Acids, *e.g.*, Butyric Acid → Acetoacetic Acid.
Conversion of Hydroxy to Ketonic Acids, *e.g.*, Lactic Acid → Pyruvic Acid.
Conversion of Amino to Fatty Acids, *e.g.*, Leucine → Isovaleric Acid.
Glycerol → Glyceric Aldehyde or Dihydroxyacetone.
*Benzene → Phenol, Catechol or Quinol.
*Glucose → Glucuronic Acid.
*Indole → Indoxyl.

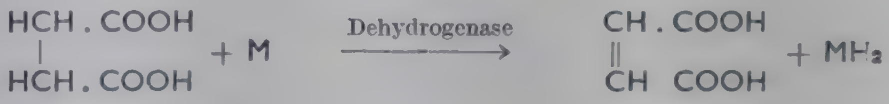
In the reactions marked * hydrogen peroxide is the only known chemical reagent which will bring about the change.

Contrary to their behaviour with other general laboratory reagents, saturated and unsaturated fatty acids are oxidised by hydrogen peroxide with equal ease, oxalic acid is only oxidised with difficulty, but malonic, succinic and glutaric acids with ease just as *in vivo*.

Guaiacum Reaction of Blood. Hæmoglobin and its iron-containing derivatives can behave like ferrous sulphate in catalysing oxidations by peroxides. This is why a blue colour is obtained on mixing guaiacum tincture, blood and hydrogen peroxide. This is not due to a peroxidase, since the reaction is given after the blood solution has been boiled. The catalytic action is due to the iron in hæmoglobin. The guaiacum reaction, therefore, is specific neither for blood nor for oxidases.

Anaerobic Oxidation

Many oxidations occur without the intervention of molecular oxygen. This is achieved by removing hydrogen ; the oxidising agent is a *hydrogen acceptor*. Transference of oxygen can be attained by the decomposition of water. The enzyme assisting the transference of hydrogen to the acceptor is a **dehydrogenase.** A very convenient hydrogen acceptor for experimental use is the dye methylene blue (M) which, on taking up active hydrogen (reduction), gives a colourless leuco-methylene blue (MH_2). A simple dehydrogenation reaction which can be easily demonstrated with methylene blue is the oxidation of succinic acid to the unsaturated fumaric acid thus :—



$$\begin{array}{l} HCH\,.\,COOH \\ | \\ HCH\,.\,COOH \end{array} + M \xrightarrow{\text{Dehydrogenase}} \begin{array}{l} CH\,.\,COOH \\ \| \\ CH\quad COOH \end{array} + MH_2$$

Succinic Acid Fumaric Acid

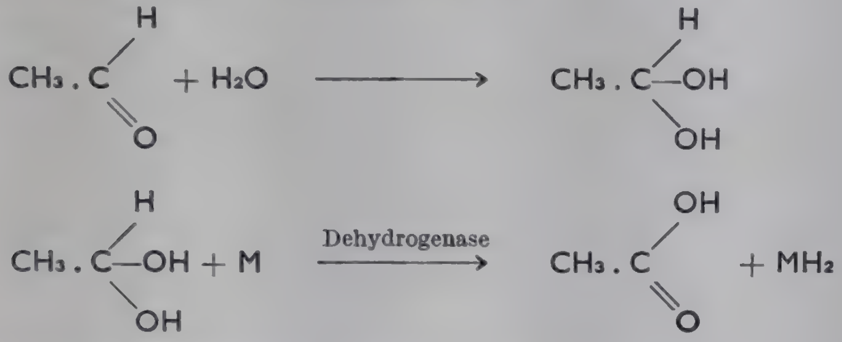
The dehydrogenase can be provided in the form of minced muscle. It should be noted that methylene blue acts as a hydrogen acceptor in the absence of air. This reaction can be generalised thus :—

$$BH_2 + X \rightarrow B + XH_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

where BH_2 is the oxidisable substance and X the hydrogen acceptor. Anaerobic oxidations are not, however, restricted to simple dehydrogenations. By inclusion of water, transference of oxygen is possible according to the general scheme :—

$$B + H_2O + X \rightarrow B.OH_2 + X \xrightarrow{\text{Dehydrogenase}} BO + XH_2 \quad . \quad (4)$$

An example of this type of reaction is



A suitable dehydrogenase is present in milk.

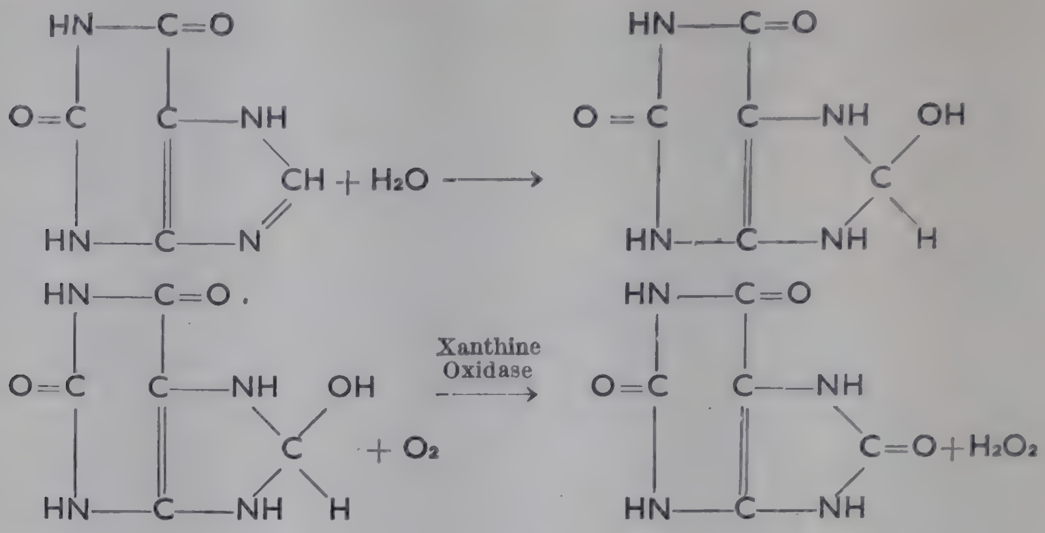
Aerobic Dehydrogenation

If a dehydrogenation is performed in air, we have the possibility of oxygen acting as hydrogen acceptor and forming hydrogen peroxide according to the general schemes :—

$$BH_2 + O_2 \xrightarrow{\text{Dehydrogenase}} B + H_2O_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

$$B.OH_2 + O_2 \xrightarrow{\text{Dehydrogenase}} BO + H_2O_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

Reduced methylene blue from anaerobic reactions (3) or (4) would be reoxidised in this way on admitting air. An example of this type of oxidation is the conversion of xanthine to uric acid, which can be represented :—



The enzyme for this reaction has long been known as xanthine oxidase. It is really a dehydrogenase.

The hydrogen peroxide formed in these reactions can be used to oxidise another substance by reaction (2). If it is not used in this way it can be destroyed by a special enzyme, **catalase,** universally distributed throughout the body. This enzyme decomposes hydrogen peroxide, but not organic peroxides thus :—

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

Molecular oxygen is evolved. Peroxidases do not give molecular oxygen, but " active " oxygen to some oxygen acceptor. Catalase cannot act as a peroxidase. Reaction (7), unlike reaction (2) with hydrogen peroxide, is not catalysed by iron.

By way of summary we may postulate the successive dehydrogenation and oxidation of a substance RH_2 by combination of the foregoing reactions, using oxygen as hydrogen acceptor thus :—

$RH_2 + O_2 \rightarrow R + H_2O_2$ by dehydrogenase, (Reaction (5))
$R + H_2O_2 \rightarrow RO + H_2O$ by peroxidase, (Reaction (2))
$2H_2O_2 \rightarrow 2H_2O + O_2$ by catalase, (Reaction (7))

The reaction could be checked at the first stage by the intervention of catalase. Note that in this case half the oxygen is regenerated.

As to the reverse process, **reduction,** these same reactions apply. The reducible substance is the hydrogen acceptor. In the above scheme, while RH_2 is oxidised, oxygen is reduced to water.

Oxidation in the Tissues

In the foregoing we have only considered purely chemical reactions with no regard to the cells in which they occur. Cell respiration is in some way related to the morphological structure of the cell. The respiration of the nucleated erythrocytes of birds is proportional to the size of the nuclei. If avian blood is hæmolysed carefully and centrifuged, only the lower layer of nuclei and cell particles respires actively. If the cells are completely disintegrated by grinding, respiration ceases. It has been shown in other tissues that normal respiration is dependent upon some structural feature of the cell. Warburg, in fact, believes that oxygen in cellular respiration is activated upon some surface in the cell where iron-containing material related to hæmatin is probably concentrated. The activation of molecular oxygen can be represented :—

$$\mathrm{Y.Fe} + \mathrm{O_2} \rightarrow \mathrm{Y.FeO_2} \quad . \quad . \quad . \quad . \quad . \quad (8)$$

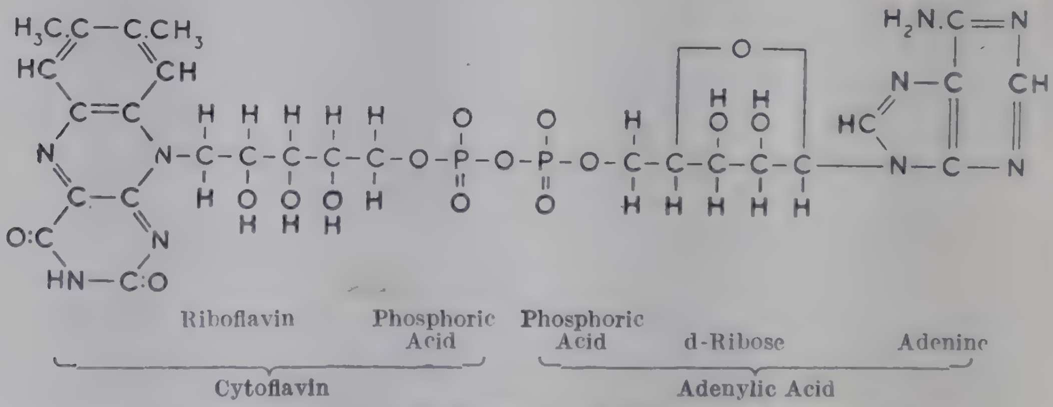
$\mathrm{Y.FeO_2}$ passes on oxygen to oxidisable substances in the tissues.

In support of this theory Warburg has shown that iron-containing charcoal, but not pure charcoal, can imitate cell oxidations. Thus leucine and cysteine solutions shaken with the charcoal at 37° C. give oxidation products similar to those obtained from intact cells. Amino-acids are not oxidised by molecular oxygen in the absence of the catalyst. As in cellular respiration, the charcoal oxidation is stopped by cyanides or certain narcotics, the former in an amount sufficient to combine with the iron ; the narcotics probably act by being adsorbed and so displacing the oxidisable substances. Peroxidases are also paralysed by cyanides and narcotics.

Amongst substances which could function as hydrogen and oxygen acceptors in accordance with the schemes described, most attention has been given to *cytochrome*, *flavoproteins*, *glutathione* and *ascorbic acid*. As to enzymes, both peroxidases and dehydrogenases are widely distributed.

Cytochrome consists of a mixture of hæmochromogen-like substances (p. 172), and is an intracellular pigment which is very widely distributed in all oxygen-using tissues. The respiratory activity of a cell is roughly proportional to its cytochrome content. Cytochrome exists in oxidised form in the presence of oxygen and reduced form in its absence. In the presence of cyanide cytochrome cannot be oxidised. This is due to poisoning of the indophenol oxidase always found associated with cytochrome. Reduced cytochrome in the presence of oxygen is converted to oxidised cytochrome by indophenol oxidase. The oxidised form, according to Keilin, is reduced again by active hydrogen liberated from tissue constituents by dehydrogenases, *i.e.*, it plays the part of the hydrogen acceptor X in reactions (3) and (4). The oxidation of cytochrome corresponds to Warburg's activation of oxygen in reaction (8). Keilin believes the system comprising molecular oxygen, oxidase, cytochrome, metabolites and dehydrogenase to form the main respiratory system of the cell. Cytochrome can also behave as a (thermostable) peroxidase for hydrogen peroxide as in reaction (2). One constituent of cytochrome (cytochrome *b*) is autoxidisable and could represent A in reaction (1).

Flavoproteins. A pigment of almost universal distribution in animals and plants is the fluorescent yellow dye riboflavin (pp. 119, 361). In the cells riboflavin is chiefly combined with phosphoric acid as a nucleotide known as **cytoflavin** (*isoalloxazine nucleotide*). Cytoflavin participates as co-enzyme with a number of dehydrogenases known as the yellow enzymes. All these dehydrogenases are composed of specific proteins united with cytoflavin although it is only present as a mononucleotide in two of these, Warburg's "*old yellow enzyme*" (the first yellow enzyme to be isolated) and *cytochrome c reductase.* In others the co-enzyme is a dinucleotide composed of cytoflavin and adenylic acid known as **isoalloxazine adenine dinucleotide.** The formula of the dinucleotide is set out below :—



ISOALLOXAZINE ADENINE DINUCLEOTIDE

The cytoflavin can accept two atoms of hydrogen on the two double-bonded nitrogen atoms. Yellow enzymes with the dinucleotide co-enzyme include *xanthine oxidase*, *diaphorase*, and *d-amino-acid oxidase*. The flavoproteins behave like methylene blue in accepting hydrogen (reactions (3) or (4)). In the reduced state they are autoxidisable giving their hydrogen to form hydrogen peroxide (reaction (5)). As an example, the "old yellow enzyme" serves in the oxidation of hexose phosphate in the following scheme :—

(a) $\text{Hexose monophosphate} + \text{Co-enzyme II} \longrightarrow \text{Phosphohexonate} + \text{Dihydroco-enzyme II}$

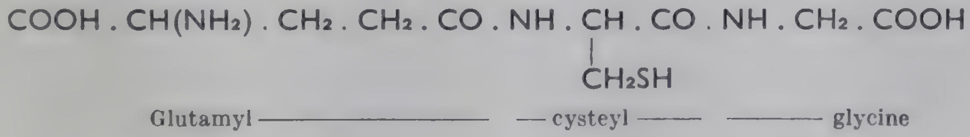
(b) $\text{Dihydroco-enzyme II} + \text{Old yellow enzyme} \longrightarrow \text{Co-enzyme II} + \text{Reduced old yellow enzyme}$

(c) $\text{Reduced old yellow enzyme} + O_2 \longrightarrow \text{Old yellow enzyme} + H_2O_2$

(d) $2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$

This scheme is a simple example of the way in which various oxidation reactions are interlinked. A number of enzymes may take part in an oxidation, hydrogen, for example, being removed from a substract and accepted by a dehydrogenase which in turn donates the hydrogen to another dehydrogenase and so on down a chain of enzymes until finally the hydrogen is accepted by free oxygen. If the oxidation of food material, *e.g.*, glucose, is carried out by chemical means *in vitro* the liberation of energy is relatively sudden, causing considerable changes in temperature and pressure. But in the body oxidation proceeds in this complicated step-wise manner with the result that, although the total energy obtained is the same, it is produced gradually, and the body can maintain the constant conditions of temperature, etc., which are necessary for its normal function.

Glutathione. This substance is almost universal in animal tissues. It is a tripeptide, glutamylcysteylglycine :—



$$\mathrm{COOH\,.\,CH(NH_2)\,.\,CH_2\,.\,CH_2\,.\,CO\,.\,NH\,.\,}\underset{\substack{|\\ \mathrm{CH_2SH}}}{\mathrm{CH}}\mathrm{\,.\,CO\,.\,NH\,.\,CH_2\,.\,COOH}$$

Freshly minced muscle tissue respires, but if thoroughly washed it no longer does so. Respiration is restored if the washings are added to the washed muscle. It had been known for some time that the ability of the washings to restore respiration was associated with the sulphydryl (—SH) content, but it was not until 1921 that Hopkins showed the essential compound was glutathione. Even after muscle tissue has been heated to 100° C., addition of glutathione restores some respiration. In glutathione the liability of cysteine to undergo autoxidation to cystine is greatly increased and we have a readily autoxidisable substance. Further, the oxidised form is an efficient hydrogen acceptor. We can abbreviate the formula of glutathione to G.SH, G representing the whole molecule except for the —SH group, and express the autoxidation thus :—

$$2G.SH + O_2 \rightarrow G.S.S.G + H_2O_2 \text{ (Reaction 5)}$$

Oxidised glutathione may act as hydrogen acceptor thus :—

$$BH_2 + G.S.S.G \rightarrow B + 2G.SH \text{ (Reaction 3)}$$

or

$$B.OH_2 + G.S.S.G \rightarrow BO + 2G.SH \text{ (Reaction 4).}$$

In the washed tissues, the oxidised substances are presumably proteins, or fatty acids from lecithin. We have here a respiratory system independent of thermolabile enzymes. Hopkins's recent work suggests that not more than 10% of normal respiration goes by this path. One function of glutathionine appears to be keeping ascorbic acid reduced.

Glutathione also plays the part of co-enzyme to glyoxalase, the tissue enzyme converting methylglyoxal to lactic acid. The reactions are probably :—

$$CH_3.CO.CHO + G.SH \rightarrow CH_3.CO.CHOH.S.G$$
$$CH_3.CO.CHOH.S.G + H_2O \rightarrow CH_3.CHOH.COOH + G.SH.$$

In view of the formation of lactic acid from glucose by erythrocytes (p. 234), it is, perhaps, significant that erythrocytes contain both glyoxalase and glutathione.

Ascorbic Acid. Ascorbic acid (vitamin C) has powerful reducing properties and is widely distributed in animal and plant tissues. It is readily oxidised by free oxygen in the presence of a suitable enzyme. The oxidation product, dehydroascorbic acid, acts readily as a hydrogen acceptor. In this way it may be concerned in oxidation-reduction reactions in the cell. One function of ascorbic acid is to keep certain substances reduced, *e.g.*, adrenaline.

From these examples it will be seen that the general reactions postulated cannot be denied on the grounds of lack of suitable reactants in the cell. The reactions in which the three chief substances are concerned and the parts they play are summarised in the table.

Reaction	Parts played by		
	Cytochrome	Flavoprotein.	Glutathione
(1) $A + O_2 \rightarrow AO_2$ . . .	A	A	—
(2) $AO_2 + B \rightarrow BO + AO$.	Peroxidase	—	—
(3) $BH_2 + X \rightarrow B + XH_2$.	X	X	X
(4) $B.OH_2 + X \rightarrow BO + XH_2$.	X	X	X
(5) $BH_2 + O_2 \rightarrow B + H_2O_2$.	—	BH_2	BH_2
(6) $Y.Fe + O_2 \rightarrow Y.FeO_2$.	Y.Fe	—	—

The relative parts played in cell respiration by these substances is not yet known.

Oxidation—Reduction Potentials (4, 12)

Whenever an oxidation occurs there must be a simultaneous reduction. The essential feature of an oxidation process is the transfer of electrons from the substance oxidised to the substance reduced. Conversely, reduction means the gain of electrons by the reduced substance. Exact electronic equations for biological oxidations would be difficult to write, but we can take as an example the oxidation of a ferrous to a ferric salt :—

$$\mathrm{Fe}^{++} \underset{\text{Reduction}}{\overset{\text{Oxidation}}{\rightleftarrows}} \mathrm{Fe}^{+++} + e$$

(The quantum e for 1 g. atom = 96,500 coulombs (1 Faraday) of negative electricity.) Oxidation and reduction in a system can be expressed generally :—

$$\text{Reductant} \rightleftarrows \text{Oxidant} + Ne$$

where N is the number of electron equivalents.

The ease with which a substance is oxidised, therefore, depends upon the readiness with which an electron is liberated. The tendency for an electron to escape is described as its **fugacity**. The greater the fugacity the greater the ease of oxidation. The electron-fugacity can be measured for any dissolved substance by finding what is called the **oxidation-reduction** or **redox potential**, E_h.

The principle of this determination is that when a strip of platinum is dipped into a solution of, say, $FeCl_2$, the electrons tending to escape will give a negative charge to the platinum. The magnitude of this charge will be proportional to the electron-fugacity. The platinum-ferrous chloride solution forms a half cell and can be combined with another half cell, such as a hydrogen electrode, as in the determination of pH (p. 16), to form a cell of which the potential can be measured. The E_h is the reading in volts when the oxidation-reduction cell is set up against a normal hydrogen electrode. A solution of an oxidising substance will oxidise substances whose solutions have a more negative E_h (*i.e.*, it will take electrons from them). A reducing substance will reduce substances whose solutions have a more positive E_h (*i.e.*, it will give electrons to them).

In a mixture we can, by determining E_h, find how much oxidised or reduced form is present. E_h is given by the following equation derived by combination of the two cells :—

$$E_h = E^0 + \frac{RT}{NF} \log_e \frac{c_{\text{oxidant}}}{c_{\text{reductant}}}$$

where E^0 is the standard *E.M.F.* of the cell, R the gas constant, T the absolute temperature, N the number of electron equivalents, and c_{oxidant} and $c_{\text{reductant}}$ the corrected concentrations of the oxidant and reductant. When the concentration quotient $\frac{c_{\text{oxidant}}}{c_{\text{reductant}}}$ is unity, $E_h = E^0$, since $\log 1 = 0$. The relation between E_h and percentage oxidation and reduction is shown in the curve, Fig. 11.

Since the curve becomes asymptotic at the ends, there is no meaning to the E_h of a pure oxidant or reductant. It is therefore convenient to express the oxidation-reduction intensity of a system at the flattest part of the curve at 50% reduction, *i.e.*, in terms of E^0. Now E^0 applies to cells where the hydrogen ion concentration is normal (pH 0). When the pH of the system is other than pH 0, the difference in hydrogen ion concentration has to be taken into account. In such systems E^0 is designated E'_o and the pH specified. E'_o, then, is the potential of the midpoint of a system at a specified pH other than pH 0. (For further details the reader must consult Refs. 4 or 12.)

Now we have already seen that dyes such as methylene blue occur in oxidised or reduced forms. If methylene blue be added to living cells it is reduced to colourless leuco-form, because methylene blue has a more positive E_h than the cells. Different dyes vary in the ease with which they can be reduced and oxidised, so that a series can be formed with graded oxidation-reduction potentials. By adding these in turn to an oxidation-reduction system we can assess its oxidation-reduction potential by the behaviour of the dye in much the same way as pH is measured by indicators. Such a method was used by Erhlich in 1885 in an attempt to measure the oxidation-reduction intensities of different tissues. He used alizarin blue and indophenol blue, which are colourless when reduced and blue when oxidised, and injected them into living animals. Later the organs were examined to see if the dyes were in oxidised (blue) or reduced (colourless) form.

Heart and brain (grey matter) did not reduce either dye, most tissues reduced indophenol blue, but not alizarin blue, whereas lung and liver reduced even alizarin blue. Nearly all tissues reduce methylene blue. Heart and brain therefore have a high oxidation potential and liver and lung a high reduction potential. The use of these dyes is not restricted to tissues. They are useful indicators for oxidation-reduction titrations. An example of biochemical interest is the estimation of vitamin C (ascorbic acid) by titration with indophenol dyes.

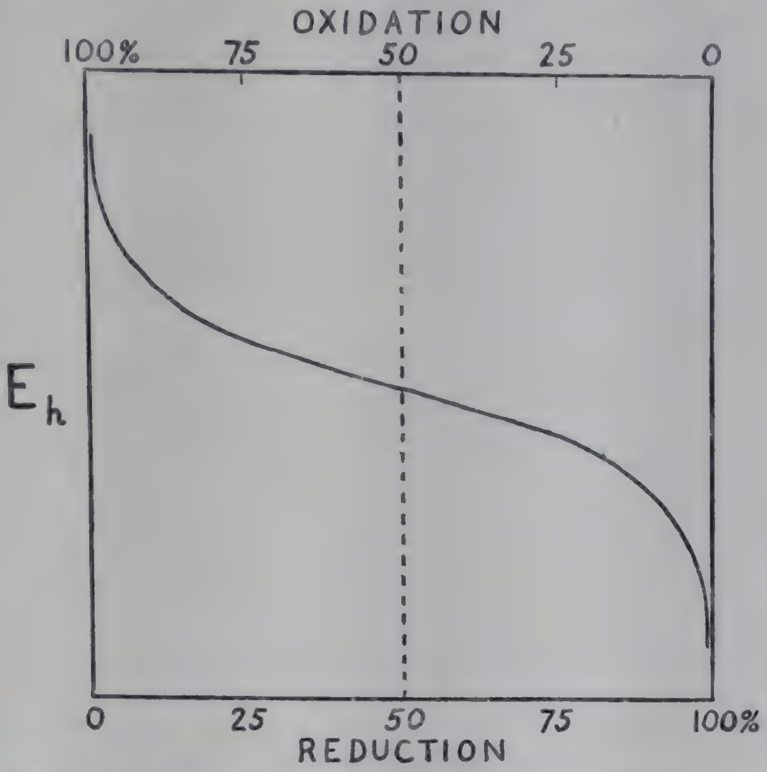


FIG. 11. Relation between E_h and percentage oxidation and reduction.

The following table gives the E'_o and E^0 values (at 30° C.) for some oxidation-reduction indicators :—

Indicator	E'_0 at pH7	E°
Phenol blue	+ 0·225	0·677
2 : 6-Dichlorophenol indophenol . .	+ 0·217	0·668
2 : 6-Dichlorophenol indo-*o*-cresol . .	+ 0·181	0·639
Toluylene blue	+ 0·115	0·601
Methylene blue	+ 0·011	0·532
Indigo trisulphonate	− 0·081	0·332
Phenosafranine	− 0·252	0·280

Dyes such as these have been used to explore the potential of large living cells such as amœbæ, the dye being injected into the cells. The results suggest that under aerobic conditions E'_0 of the intracellular contents is $-$ 0·07 and under anaerobic conditions $-$ 0·3 at pH 7. It has already been mentioned that most tissues reduce methylene blue, *i.e.*, their E_h is less than $+$ 0·011.

The oxidant of adrenaline in neutral solution is extremely unstable. The E'_0 of the system at pH 7·16 is $+$ 0·345, *i.e.*, it is so positive that the tissues can keep adrenaline in the reduced state, and so prevent its destruction.

Not all oxidation-reduction systems have the reversibility of those we have been considering. Almost instant equilibrium may be attained and maintained, the presence of a catalyst may be essential to the attainment of equilibrium, or one component may be decomposed or removed as soon as it is formed. Consequently not all oxidation-reduction reactions can be recorded in terms of E'_0.

CHAPTER XII

BLOOD (1, 3, 4, 5, 8, 10)

In spite of more attention having been devoted to a study of the chemistry of blood than that of any other tissue, we are still unable to claim a complete knowledge of its composition, either qualitative or quantitative. On the basis of the identified constituents alone, blood is an extremely complex fluid ; yet it is certain that other constituents remain to be identified. When the many functions of blood are considered, this is not surprising. The chief functions of blood can be enumerated as follows :—

(1) The transport of oxygen from the lungs to the tissues.

(2) The transport of carbon dioxide from the tissues to the lungs.

(3) The transport of substances, absorbed from the intestine, to the tissues.

(4) The transport of waste products of metabolism to the organs of excretion—kidneys, skin, intestine and lungs.

(5) The transport of substances from one tissue to another.

(6) The transport of the chemical regulators of metabolic processes—the hormones and vitamins.

(7) By its efficient buffering power and in conjunction with the kidneys and lungs it maintains the acid-base equilibrium of the body.

(8) Assisted by the kidneys and the skin, it maintains a steady osmotic pressure in the tissues and fluids of the body.

(9) Owing to the plasma proteins, it takes part in the regulation of the water balance of the tissues.

(10) In conjunction with the skin, it is concerned in the maintenance of body temperature at a constant level.

(11) By clotting it guards against hæmorrhage.

(12) The white corpuscles form a defence against bacteria.

(13) The blood contains other substances which combat toxic agents ; they include antitoxins, agglutinins, precipitins.

Most of these functions will not be discussed in this chapter; some are fully described in the standard text-books of Physiology (6, 10, 11) and of Pathology and Bacteriology (12, 13), to which the reader must be referred; the rest are described in appropriate places (see index) in this book.

Properties of Blood

Freshly shed blood is a red opaque slightly alkaline fluid; it is slightly viscous and sticky to feel; it has a salt taste and characteristic smell. It consists of a suspension of cells (corpuscles or formed elements) of various types in a clear straw-coloured liquid—**plasma.** The cells in man form about 45% of the total volume of the blood; this ratio is probably not uniform throughout the circulation. The most abundant cells are the **erythrocytes** (**red cells** or **red corpuscles**) (about 5,300,000 per cu. mm. in man); there are also the **blood platelets** (**thrombocytes**) (about 300,000 per cu. mm., but very variable) and the **leucocytes** (**white cells** or **white corpuscles**) (about 10,000 per cu. mm.). The leucocytes consist of several different types of cells (see text-books of Histology). In biochemical investigations blood is usually regarded as consisting of red cells and plasma, since this is the only simple separation for analytical purposes, although in some instances we do not even know whether a substance is carried by the blood in the plasma or in the cells. That the white cells or platelets may be predominantly concerned in the carriage of certain substances is evidenced by histamine, which is almost entirely confined to these cells in blood (Code and Ing).

Clotting of Blood. Freshly shed blood, on standing, rapidly undergoes changes, first becoming more viscous and finally setting to a red jelly. This change, which is known as the "**clotting**" or "**coagulation**" of blood, usually occurs within five or ten minutes after shedding, even if the blood is left undisturbed. There are, however, many factors which affect the clotting time (coagulation time), including the temperature and the nature of the vessel in which the blood is collected. If the blood be collected in vessels previously coated with paraffin or oil, and if dust is carefully excluded, clotting may be considerably delayed.

Clotting may be accelerated by immersing in the blood substances which are wetted by it, *e.g.*, threads of cotton or wool, a fact applied in stopping bleeding from a cut. The process of blood clotting is a complicated one, and, since it is described at length in all text-books of Physiology, will only be summarised here. The fundamental process is probably as follows :—

An activator, **thrombokinase** (which some think identical with kephalin, see p. 81), is formed, either by breakdown of platelets on contact with a surface which blood wets, or in damaged tissues. Thrombokinase causes the conversion of a substance **prothrombin** (prothrombase) present in blood into an enzyme-like substance, **thrombin** (thrombase). **Calcium ions** are essential for a rapid conversion. Thrombase acts upon a soluble plasma protein, **fibrinogen,** to produce an insoluble protein, **fibrin,** which separates as fine threads. The clot consists of a network of these threads in which the corpuscles and residue of plasma are entangled. The details of the stages and the exact nature of the substances mentioned are much disputed. The clotting process is independent of the corpuscles. Plasma alone will clot provided thrombokinase is present (*e.g.*, in a tissue extract).

If the red jelly first formed on clotting is left to stand for some hours, the network of fibrin contracts and squeezes out the plasma residue or **serum** as a straw-coloured liquid. Serum is plasma without fibrinogen.

If freshly shed blood is briskly stirred (" whipped ") with a bunch of twigs or wires, the fine fibrin threads are caught up on the stirrer forming thick stringy threads, and so removed as they are formed. In this way the whole of the fibrin can be removed, leaving a blood, **defibrinated blood,** which does not clot and is composed of corpuscles + serum. On standing, the corpuscles gradually settle, leaving serum as the supernatant layer. The separation of corpuscles and serum is more rapidly achieved by centrifuging.

Prevention of Clotting. Since blood is especially liable to clot in narrow tubes such as pipettes, the analysis of whole blood or plasma is only practicable if the clotting is prevented. Of the many methods known to physiologists only two are commonly used for analytical purposes. The most convenient is to remove calcium ions from the blood by adding salts such as potassium oxalate or citrate, preferably the former, since a relatively large amount of citrate is required. The plasma formed by centrifuging blood so treated is called **oxalated** or **citrated plasma.** Oxalated blood (or plasma + thrombokinase) will clot if calcium ions are added in excess of the oxalate. If added in the proper amount (3 mg. per c.c. of blood) and under suitable conditions to ensure rapid mixing, potassium oxalate is an effective anti-coagulant which does not appreciably affect the distribution of water and electrolytes in blood, so that it can be used for most analyses of either whole blood or plasma. The other method of preventing clotting of a sample for analysis is defibrination. By stirring with a glass rod with certain precautions, fibrin can be removed without appreciable alteration in the other serum constituents, and serum can be obtained suitable for analysis of Ca, K, Na, etc., although where time and conditions permit it is more usual to obtain serum by allowing the blood to clot overnight. The use of heparin as an anti-coagulant may become practicable when cheaper and purer specimens are available.

Osmotic Pressure. The depression of freezing-point, $\triangle$, of human blood exposed to air is about 0·53° C., which is the same as that of 0·9% NaCl. This is equivalent to an osmotic pressure of 6·9 atmospheres (assuming complete dissociation). Recent measurements (Margaria) of the vapour pressure of blood in equilibrium with 5% CO_2 have shown that the average osmotic pressure of the blood of eighteen men is equal to 0·945% NaCl, and that of sixteen women 0·927%. Normally the osmotic pressure of blood is very constant. Ingestion of large amounts of water reduced it slightly, whereas strenuous exercise increased it, the extreme values observed being equivalent to 0·882% and 1·048% NaCl respectively.

The sodium chloride in plasma accounts for most of the osmotic pressure of blood. The **colloid osmotic pressure** (**oncotic pressure**), that is, the osmotic pressure of the non-dialysable constituents, is mainly due to the plasma proteins and is equivalent to a pressure of about 28 mm. of mercury (= 380 mm. of water). Although small, this colloid osmotic pressure is important, since it probably provides at least part of the driving force necessary for the transfer of fluids through membranes in the body (p. 159).

pH of Blood. Blood is slightly alkaline in reaction and its pH is normally between pH 7·3 and 7·5. (As ordinarily used, the term blood pH really means *plasma pH* ; the pH of the cells is about 7·1.) In the resting individual, arterial blood is very slightly more alkaline (about 0·02 pH) than venous. This difference is increased by muscular exertion due to the increased amount of acid (carbonic and lactic) formed by the muscles. An individual with a blood pH below 7·3 is described as being in a condition of **acidosis,** and over pH 7·5 **alkalosis.** Under normal conditions the blood pH is maintained in the region of pH 7·4, in spite of the continual addition of carbonic acid, due, as explained in Chapter II, to the efficient buffering of the blood. A further important defence against acidosis is the power of the kidneys to excrete more acid phosphate, BH_2PO_4, than alkaline, B_2HPO_4 (p. 298), and to form ammonia from urea (p. 273).

The cause of an *abnormal* pH may be either *metabolic*, due primarily to deficit or excess of alkali relative to acids other than carbonic, or *respiratory*, due primarily to deficit or excess of carbonic acid. *Acidosis* (low pH, alkali deficit) would result from (*a*) fixation of base by abnormal acids, *e.g.*, acetoacetic and β-hydroxybutyric acids in faulty fat metabolism (starvation, diabetes mellitus, p. 254), (*b*) failure to excrete NaH_2PO_4, *e.g.*, in chronic nephritis, (*c*) administration of acidic or acid-producing substances, *e.g.*, NH_4Cl, or (*d*) failure to remove CO_2, *e.g.*, in breathing CO_2 rich air or in lung diseases. *Alkalosis* (high pH, alkali excess) might be due to (*a*) loss of acid, *e.g.*, of HCl by severe vomiting, (*b*) retention of alkali after administration of alkali, *e.g.*, $NaHCO_3$, or (*c*) loss of CO_2 by overbreathing, whether due to oxygen lack, *e.g.*, at high altitudes or in heart disease, or in an attempt to reduce temperature in fevers.

Specific Gravity. The specific gravity of normal blood lies between 1·041 and 1·067 and usually between 1·05 and 1·06. That of plasma lies between 1·024 and 1·038 (water = 1·000) and is roughly proportional to the protein content.

Viscosity. The viscosity of blood is important, since it determines the resistance to the flow of blood through the capillaries and smaller vessels, and hence the blood pressure. (The diameter of the vessels is, of course, also significant.) Human blood is about five times as viscous, or as "thick," as water. This value is determined by timing the flow of a given volume of blood through a specially shaped capillary tube, *viscosimeter*, and comparing the time with that taken by the same volume of distilled water. An anti-coagulant must be added to the blood previous to the determination.

The high viscosity of blood is mainly due to the cells. Plasma or laked blood (blood in which the cell membranes have been

Normal Values for Adult Human Blood in Mg. per 100 c.c. unless otherwise Stated

	Whole Blood	Plasma or Serum	Remarks
Acetone bodies (as acetone)	1–3	—	—
Albumin	—	3·4–6·7%	% = g. per 100 c.c.
Alkali reserve	—	50–77 vols. CO_2 per 100 c.c.	—
Amino-acid nitrogen	3–8	4–6·5	—
Ammonia	0·1–0·25	0·1–0·25	—
Bicarbonate	—	53–77 vols. CO_2 per 100 c.c.	—
Bile acids	2·5–6·0	5–12	—
Bilirubin	—	0·1–0·5	—
Calcium (total) (Ca)	5–7	9–11	See p. 301.
Carbon dioxide in physical solution at 40 mm.	—	2·5–4·5	—
Chloride (NaCl) (Cl)	450–530 270–320	560–620 340–380	— —
Cholesterol (total)	100–200	100–200	—
Corpuscular volume	36–51%	—	—
Creatine	2–9	0·5–3	—
Creatinine	0·7–2	0·7–2	—
Fatty Acids	290–420	190–640	—
Fibrinogen	110–210	200–400 (In plasma)	—
Fragility range	0·35–0·45% NaCl	—	± 0·03%
Freezing-point	– 0·51 to – 0·62° C.	– 0·51 to – 0·62° C.	Average – 0·56° C.
Globulin	—	1·2–2·9%	—
Glucose, see Sugar.			
Glutathione	25–50	0	Reduces alkaline copper solutions.
Hæmoglobin	13–16% (Men) 95–120 " per cent." *	0	Women, 12–14% 88–102 " per cent." *
Iron (Fe)	40–55	0·06–0·2	—
Lactic acid	5–35	—	May rise to 200, p. 236
Lecithin (lipide P × 25)	250–450	170–350	—
Lipides (total)	200–2,000	450–1,260	" Total ether-soluble matter."
Magnesium (Mg)	2–4	1–4	—

* (100 " per cent." (Haldane) = 13·8% = 18·5 c.c. O_2 combining power.)

Normal Values for Adult Human Blood—*continued*

	Whole Blood	Plasma or Serum	Remarks
trogen.			
Free in solution . .	1·3 c.c. per 100 c.c.	—	—
Total N	2·6–4·3%	1·1–1·4%	Mainly protein N.
Non-protein N	25–50	18–30	—
Amino-acid N . . .	3–8	4–6·5	—
Ammonia N . . .	0·1–0·2	0·1–0·2	—
Creatine N	0·6–2·9	0·2–1·0	—
Creatinine N	0·3–0·7	0·3–0·7	—
Urea N	7–20	7–20	—
Uric acid N	0·1–1·3	0·1–1·3	—
ygen.			
Combining power . .	16–24 c.c. per 100 c.c.	—	Women lower.
Content, arterial . .	15–23 ,, ,, ,,	—	,,
,, venous . .	10–18 ,, ,, ,,	—	,,
Free in solution . .	0·1–0·2 ,, ,, ,,	—	—
Tension, arterial . .	84–100 mm.	—	—
,, venous . . .	30–60 ,,	—	—
enols	2–8	—	—
osphorus.			
Total P	28–48	6–18	—
Inorganic P	2–5	2–5	Children, 4–6.
Ester (organic) P. . . .	14–29	0–4	—
Lipide P	8–18	3–14	—
tassium (K) . . .	150–250	18–21	—
oteins.			
Plasma	—	5·8–8·6%	See pp. 157–161.
Serum	—	5·6–8·5%	
Albumin	—	3·4–6·7%	
Globulin	—	1·2–2·9%	
Fibrinogen	—	0·2–0·4%	
Albumin : Globulin Ratio .	—	4 : 1–1·2 : 1	Usually 2 : 1.
efractive index (17·5° C.) .	—	1·349–1·351	Water = 1·333.
dium (Na)	170–225	325–350	—
lids (total)	18–25%	8·5–10%	—
G.	1·041–1·067	1·024–1·038	Water = 1·000.
gar.			
Fasting	60–120	60–120	Reducing value.
Max. after meal	180	180	True glucose less, p.220.
lphur.			
Total non-protein S . .	3·8–5·1	3·11–3·86	To get SO_3 multiply by 2·5.
Inorganic S	0·28–0·65	0·50–1·12	
Ethereal S	0·07–0·96	0·09–0·96	
Neutral S	3·19–4·32	1·72–2·54	
hioneine (Ergothioneine) .	0–5	0	Contains 14% S.
rea	15–40	15–40	—
ric acid	0·3–4·0	0·3–4·0	—
robilin	—	0·0–0·4	—
iscosity (20° C.) . . .	3·6–5·6	1·7–2·0	Water = 1·0.
olume	3·5–7 litres 6–12 pints	2–4 litres 3·5–7 pints	— —
eight.			
Percentage body weight .	7–9	4·3–5·7	—
Fraction body weight . .	$\frac{1}{14}$–$\frac{1}{11}$	$\frac{1}{23}$–$\frac{1}{18}$	—

The values in this table are, with few exceptions, quoted from Harrison, *Ref.* 3, pp. 333–336.

ruptured and their contents dissolved in the plasma) has a very much lower viscosity. The relative viscosities of water, plasma and whole blood are 1, 1·7 to 2 and 3·6 to 5·4. The viscosity of blood may be affected by any factors causing alteration in the numbers or size of red or white cells. Abnormal values are observed in polycythæmias, leukæmias (high), severe hæmorrhage and pernicious anæmia (low).

One reason why injection of gum saline is more effective than injection of saline for making up the blood volume after severe hæmorrhage is that the high viscosity of the former, by its resistance to flow through the smaller vessels, enables blood pressure to be better maintained.

Volume. A method of measuring blood volume to meet universal acceptance has yet to be discovered. All the present methods, which really estimate the volume of either plasma or red cells, are open to the criticism that it is assumed that the ratio of cells to plasma is the same throughout the body. It is usually stated that the quantity of blood is about 8% or 9% (one-twelfth or one-eleventh) of the body weight, so that a man of 70 kg. (11 st.) would have about 6 litres of blood.

Composition of Blood

The table on p. 154 gives the range of values for the commoner constituents of normal human blood and plasma. In most instances there is considerable variation in different healthy individuals, although the majority yield values near the centre of the range. It is misleading to think of average values as applying to every healthy individual, except in certain constituents with a narrow range, *e.g.*, calcium. A selection of the more important constituents whose values should be memorised is given in the Appendix. It will be realised that many values, *e.g.*, sugar, amino-acid N, fat, will rise shortly after a meal and subsequently fall to a fasting level. It is obvious from the figures given that some substances, *e.g.*, sugar and urea, are fairly equally distributed between plasma and corpuscles, whereas others are almost entirely confined to one or the other, *e.g.*, Na and Ca in plasma and K in corpuscles.* The majority of substances in the table are discussed either in the following pages or in other chapters (see index).

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* This is not true for all bloods. In the dog, for example, Na and K are distributed approximately equally between corpuscles and plasma.

Substances circulating in blood not mentioned in the table include bromide, iodine, indican, enzymes, anti-enzymes, hormones, vitamins and various " immune bodies."

PLASMA AND SERUM

Plasma contains 8% to 9% of solids, composed largely of coagulable proteins. The constituents of plasma are distributed roughly as follows :—

Proteins	7·0%
Lipides	0·7%
Other organic substances	0·15%
Inorganic substances	0·75%

Proteins. The plasma proteins in normal individuals may vary from 6·0% to 8·5%. In certain types of nephritis with œdema in which there is a heavy albuminuria, values of 5% or lower may be observed. The proteins are usually stated to be of three types, distributed approximately as follows :—

Serum albumin	4·5% of plasma.
Serum globulin	2·2% " "
Fibrinogen	0·3% " "

(About 0·1% of a glycoprotein, seromucoid, is also present.)

It would be more correct to say that plasma protein can be separated artificially into three parts corresponding to the above. It is highly probable that in the living animal there is only one plasma protein which is a labile complex unit dissociated into commonly isolated proteins by physico-chemical treatment such as salting out. The amounts of these proteins formed depend upon the technique of isolation employed. The *purified* isolated proteins can be regarded as chemical entities. Studies of the sedimentation of serum in the ultra-centrifuge indicate at least three " molecular " fractions, corresponding to albumin, globulin and an intermediate protein, but the ratio of albumin to globulin is about 4 : 1, instead of 2 : 1 as found by salting out with ammonium sulphate. Dilution of the serum with isotonic saline gives values nearer to 2 : 1. Ultrafiltration of serum through graded membranes suggests the existence of albumin and globulin fractions, the latter existing in a state of aggregation which dissociates on dilution. Chemical analysis of the total serum proteins reveals an arginine : lysine ratio of 10 : 18, which is constant even when the albumin : globulin ratio, as determined by salting out, has been altered in disease, *e.g.*, nephrosis. The lysine content of separated fractions differs widely according to the technique employed ; the arginine content is fairly uniform. The name **orosin** has been suggested for the total coagulable protein of serum. All mammalian orosins have an arginine : lysine ratio of 10 : 18.

The foregoing does not invalidate any clinical observations based upon the *albumin-globulin ratio* of sera, provided the values are determined, as they usually are, under comparable conditions. The albumin : globulin ratio of normal serum is usually about 2 : 1 ; in nephrosis it may fall to 0·5 : 1 or less.

Whatever the true nature of the proteins of serum, recent work leaves no doubt that we must consider them as a labile system in a delicately balanced equilibrium and not as several distinct and stable chemical individuals mixed together in solution. The subsequent descriptions of serum proteins apply to the substances which have been separated from the serum.

Serum Albumin. The separation of serum proteins into albumins and globulins can be effected by half-saturation with ammonium sulphate by which globulins are precipitated, while the albumins remain in solution and are only precipitated by fully saturated ammonium sulphate. The albumin of serum is probably a mixture of several albumins, even after it has been crystallised. It is soluble in distilled water. The molecular weight is about 68,000.

Serum Globulin. The globulins precipitated by half-saturated ammonium sulphate can be further separated into **euglobulin,** precipitated by one-third saturated ammonium sulphate, and **pseudoglobulin,** precipitated by half-saturation. Euglobulin is insoluble in water and only soluble in the presence of salts. Pseudoglobulin is soluble in water unless the last traces of salts are very carefully removed ; it is difficult to free it from albumin. Chick attributed the difference between euglobulin and pseudoglobulin to the presence of phosphatides in the former. The molecular weight of serum globulin has been given as 150,000 (ultra-centrifuge method) and 175,000 (osmotic pressure method). The two methods are in agreement for serum albumin.

The site of the formation of albumin and globulin is uncertain. After depletion (*e.g.*, by hæmorrhage) these proteins are regenerated to the normal level in about fourteen days. In diseases involving albuminuria (*e.g.*, types of Bright's disease) as much as 25 g. per day may be excreted continually, and yet a constant plasma protein level is maintained, so that albumin can be regenerated at least at this rate. After hæmorrhage, globulin is regenerated more quickly than albumin. Both these proteins are regenerated slowly compared with fibrinogen (see below).

Fibrinogen. The protein which distinguishes plasma from serum has the characteristics of a globulin which is unusually easy to precipitate. It is coagulated by heat at about 56° C. (serum globulin about 70° C.) and precipitated by one-fifth saturation with ammonium sulphate. It is distinguished from the other plasma proteins by its property of clotting. Fibrinogen is either formed in the liver or its production controlled by the liver, since removal of that organ (or severe liver disease) is followed by a progressive decrease in fibrinogen. When fibrinogen is removed from an animal experimentally (*e.g.*, by replacing its blood by defibrinated blood or washed corpuscles suspended in Ringer-Locke solution), it is rapidly regenerated in a few hours provided the liver is intact. Tissue injury and inflammation stimulate fibrinogen production.

Fibrin. The protein formed from fibrinogen by "whipping" blood forms stringy threads which can be washed free from red cells, but are usually contaminated by fragments of platelets and cells. A purer fibrin is obtained by clotting separated plasma. The fibrin which first forms on clotting appears (under the microscope) as microcrystalline needles. Apart from the obvious one of solubility, there is no definite chemical difference between fibrinogen and fibrin ; it has even been suggested that the change is mainly a physical one of the non-reversible "sol-gel" type (*e.g.*, egg-white, p. 44). The difficulty is that there are as yet no criteria of purity for either fibrinogen or fibrin ; it will be recalled that clotting is associated with substances such as calcium, kephalin (?), thrombase, which invariably contaminate the clot.

Functions of Plasma Proteins

Apart from the important *rôle* of fibrinogen in blood clotting, the principal function of plasma proteins is that of regulating the passage of water between blood and the tissues. The colloid osmotic pressure (oncotic pressure) of plasma is about 28 mm. of mercury. Blood and the tissues are approximately isosmotic with respect to their crystalloids, so that there is an osmotic attraction of water from the tissues into the blood, since the oncotic pressure of the tissue proteins is less than that of the plasma proteins. In opposition to this water attraction, the blood pressure provides a filtration pressure in the capillaries by which water tends to pass from the blood to the tissue spaces. The hydrostatic pressure at the arterial end of the capillaries is slightly greater (32 mm. Hg in human skin) and at the venous end less (12 mm.) than the colloid osmotic pressure, so that normally there is an equilibrium between the opposing forces. Interference with this equilibrium will result in increased water either in the blood or in the tissues, as under the following conditions :—

(1) If the capillary pressure is increased as by venous obstruction, more water will flow into the tissues, since the hydrostatic pressure is greater than the oncotic pressure.

(2) A fall in blood pressure, as after hæmorrhage, will assist the passage of water from the tissues into the blood.

(3) If the plasma proteins are decreased, water will flow into the tissues, since the opposing osmotic force is decreased. *The osmotic pressure of the albumin of plasma is not the same as that of the globulin.* Since osmotic pressure is dependent on the number of molecules per unit volume, serum albumin with its smaller molecular weight will have a greater osmotic pressure per unit weight than serum globulin, which has a larger molecule. The albumin in serum exerts an osmotic pressure of about 7·5 cm. water (5·5 mm. Hg) per gram of albumin and the globulin about 2 cm. (1·4 mm. Hg).*

Consequently the albumin : globulin ratio is important as well as the total amount of plasma protein. In albuminuria, albumin having the smaller molecule escapes in the urine more readily than globulin, so that the colloid osmotic pressure will be lowered not only by a fall in total protein, but also by a fall in the albumin : globulin ratio.

(4) Dilution of blood by excessive ingestion of water, or intravenous injection of aqueous solutions, will lower the osmotic pressure of the plasma proteins and may also increase capillary pressure, so that two factors will operate in the rapid removal of excess water from the blood into the tissues.

The condition of œdema (abnormal accumulation of fluid in the tissue spaces) may be caused by a number of factors, which are described in text-books of Applied Physiology or Pathological Chemistry. It usually occurs if the colloid osmotic pressure falls below 250 mm. H_2O, total proteins below 3%, or albumin below 1·5%. If the plasma proteins are considerably reduced (*e.g.*, to 1%), fatal shock ensues.

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* These are values determined experimentally. Theoretically, on the basis of their molecular weights, the difference should be less. The discrepancy has not been explained.

Another important function of plasma proteins is their buffering action. Since the isoelectric points of these proteins are acid (albumin pH 4·7, globulin pH 5·4, fibrinogen pH 5·0–5·5), they will be present in blood as salts of weak acids and act as buffers. According to van Slyke, the base held in combination by 1 litre of plasma (70 g. protein) is equivalent to 17 c.c. N.NaOH. Since this base is available for combining with CO_2, it forms a valuable part of the blood " alkali reserve " and aids the transport of CO_2.

The plasma proteins contribute to the viscosity of blood (p. 152), the viscosity of plasma (1·8) being almost entirely due to the proteins.

The plasma proteins normally appear to have no direct nutritive function. Fasting for limited periods does not affect the serum protein level. By prolonged deprivation of protein (and in equivalent wasting diseases) the serum protein (mainly albumin) is lowered and may lead to nutritional œdema.

Other Constituents of Plasma

The substances of low molecular weight found in plasma can mostly be classed, either as substances with a primarily nutrient function (*e.g.*, glucose, amino-acids, fats, salts, oxygen) in transport to the tissues, or as waste products of metabolism (*e.g.*, urea, CO_2, and in part creatinine, uric acid, certain salts).

The crystalloid (mainly salts) content of plasma is maintained approximately isosmotic with that of the tissues largely by means of the kidney.

The colour of plasma is due chiefly to small amounts of bilirubin and carotene and related pigments.

According to Peters and van Slyke, the distribution of basic and acidic radicles in milliequivalents (mg. per litre/equivalent weight in mg.) is approximately as follows :—

Base $Na^{\cdot}$ 142, $K^{\cdot}$ 5, $Ca^{\cdot\cdot}$ 5, $Mg^{\cdot\cdot}$ 3 = 155
Acid Cl' 103, HCO_3' 28, PO_4''' 2, SO_4'' + organic 6, protein 16 = 155

LEUCOCYTES

Leucocytes have no chemical feature distinguishing them from typical animal cells. They contain the usual constituents which include proteins, nucleoproteins, fats, lecithin, cholesterol, purines, enzymes and inorganic salts. They are well supplied with proteases. The difficulty of obtaining leucocytes in sufficient quantity precludes frequent analysis. The separation of the different types of leucocytes for analysis would be almost impossible.

BLOOD PLATELETS

Little information is available as to the chemical nature of blood platelets.

RED BLOOD CORPUSCLES

The average diameter of a human * red blood corpuscle is about 8·8 μ (in a blood film, owing to shrinkage, the diameter is about 7·5 μ), and its surface area about 0·00013 sq. mm. Since there are about 5,000,000 per cu. mm. of blood, the total surface area of the corpuscles in blood is very large (about 600 sq. metres per litre). This is of great importance in facilitating the interchange of substances (especially oxygen) between corpuscles and plasma. Chemically it is convenient to picture a red cell as essentially a droplet of a red sol enclosed in a membranous bag, although no limiting membrane has been demonstrated histologically. Such a membrane is probably formed by surface concentration of a molecular layer of protein and lipide (lecithin-cholesterol). The characteristic biconcave shape of a red cell is ascribed to the presence of a framework (*stroma*) of protein ; it has, alternatively, been suggested that the red corpuscle is not a cell in the usual sense, but that its form and identity are maintained because its constituents exist in the gel state. Whatever the structure, the red cell is flexible and can adapt its shape temporarily in passing through small capillaries ; the cell boundary behaves like a membrane which is normally impermeable not only to colloids but to many crystalloids, but readily permeable to solutions of gases. Under certain conditions the continuity of the "membrane" is interrupted (or the "gel" state is upset) and the cell contents mix with the plasma or serum. This process is called *hæmolysis* or *laking.*

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* The corpuscle size varies in different mammals between 2 μ (musk deer) and 10 μ (elephant).

Hæmolysis

Destruction of red cells by hæmolysis occurs both *in vivo* and *in vitro.* Blood may be hæmolysed in several ways :—

(1) **Osmotically.** Since the red cell membrane is permeable to water, the volume of the cell changes according to its osmotic environment. When placed in a hypotonic solution, red cells swell owing to water passing in (**endosmosis**). If the solution is sufficiently hypotonic, so much water is taken in that the cells become spherical or even "ruptured," that is, the continuity of the "membrane" is interrupted, probably from lack of sufficient lipide to form a continuous layer over the increased surface, and the cell contents diffuse into the surrounding fluid. The stroma of the cell is not destroyed, and can be seen (the so-called "ghost") under the microscope with dark ground illumination. If cells are placed in a hypertonic solution, water passes out of the cells (**exosmosis**) and they shrivel, due to diminution in volume. This process is called **crenation.**

The resistance of erythrocytes to hypotonic solutions has clinical application in the **fragility test** ; a drop of blood is carefully mixed in each of a series of tubes with hypotonic NaCl solutions graded by 0·05% and the extent of hæmolysis observed in each tube after standing about two hours. If no hæmolysis occurs there is a colourless supernatant layer over an opaque red suspension of cells ; if hæmolysis is complete, a transparent red solution is seen. In normal human blood complete hæmolysis occurs only below about 0·35% NaCl ; the cells resist hæmolysis above 0·45% ; in between there is partial hæmolysis, due to variations in the resistance of individual cells. Diminished resistance (increased fragility) is seen in hæmolytic or acholuric jaundice (up to 0·7–0·8%) and increased resistance in certain anæmias.

Osmotic hæmolysis has been produced experimentally *in vivo* by injection of a large volume of distilled water and even very excessive (forced) ingestion of water.

(2) **By Hæmotoxins or Hæmolysins.** Various biological agents such as snake venom, hæmolytic streptococci and certain other bacteria can produce hæmolysis *in vivo.* The sera of certain other species of mammals are also able to produce hæmolysis *in vivo* ; the Wassermann reaction for syphilis employs a hæmolytic

system, produced by the injection of foreign red cells into an animal such as a rabbit.

Hæmolysis is encountered clinically in (*a*) hæmolytic anæmias and jaundice, jaundice of the new-born, or (*b*) blackwater fever, paroxysmal hæmoglobinuria and conditions covered by (2). In the normal breakdown of old red cells (see p 174) and in group (*a*) which includes conditions of abnormal destruction of red cells, the reticulo-endothelial system rapidly removes the hæmoglobin and converts it to bilirubin, so that no hæmoglobin is observed in the plasma. In group (*b*) the reticulo-endothelial system is overtaxed with the result that there is hæmoglobinæmia.

(3) **By certain drugs** *in vivo, e.g.*, quinine, phenacetin, nitrites and chlorates.

(4) **By Lipide Solvents.** The fat solvents, *e.g.*, alcohol, chloroform, ether, and substances like soaps, bile salts, saponin, which form soluble compounds with lipides, cause hæmolysis, due either to their solvent action on the lipide "membrane," or to their power of lowering surface tension and so interfering with the "membrane."

(5) **By Mechanical Means.** Grinding, vigorous stirring or shaking is liable to cause destruction of erythrocytes. The hæmolysis of blood by alternate freezing and thawing is probably essentially an osmotic hæmolysis due to local hypotonicity near the melting ice crystals.

(6) **Other hæmolytic agents** *in vitro* include heat, pH changes, ultra-violet rays.

Composition of Red Corpuscles

The red corpuscles contain 32% of solids, a higher proportion than other soft tissues; of these solids the greater part is the red protein, hæmoglobin. The following is the approximate composition :—

Hæmoglobin	29%
Other proteins (mainly of stroma) . .	0·5–1·0%
Lipides	1·0%
Other organic substances . . .	0·2%
Inorganic substances	0·7%

The lipides are mainly concentrated in the "membrane"; the non-hæmoglobin protein is mainly in the stroma, but its exact nature is uncertain. The main interest lies in the red pigment, hæmoglobin, owing to the part it plays in the carriage of O_2 and CO_2 in respiration.

HÆMOGLOBIN

\The red pigment, hæmoglobin, is a conjugated protein. Its molecular weight is about 68,000. It contains four atoms of iron and is probably an association of four molecules of M.W. 17,000; its molecular formula approximates to $(C_{712}H_{1130}O_{245}N_{214}S_2Fe)_4$. The iron content is about 0·34%. Although a protein, hæmoglobin from many species (*e.g.*, dog, guinea-pig, rat. horse) can be very easily crystallised, often by just cooling the laked blood; the hæmoglobin of other species crystallises with greater difficulty. The form of the crystals, their solubility and the ease of crystallisation are characteristic of the species (or closely related species) from which the hæmoglobin is obtained. The crystals are most easily obtained from oxygenated blood, *i.e.*, crystals of oxyhæmoglobin. Most bloods (including human) form rhombic prisms or needles; the bloods of the guinea-pig and squirrel are distinctive in giving tetrahedra and hexagonal plates respectively. The hæmoglobins of different species are said to be immunologically distinct. The difference must lie in the protein moiety, globin, since the red prosthetic group is identical in all species; the amino-acid composition of different globins varies slightly, especially in respect to cystine. The hæmoglobins of different species also have different affinities for combining with oxygen. In man there are probably at least two hæmoglobins, a fœtal and an adult hæmoglobin.\

Combination with Gases. The most characteristic and physiologically important property of hæmoglobin is the ease with which it combines with oxygen and dissociates again. On exposure to air or oxygen a solution of hæmoglobin readily combines with 1·34 c.c. O_2 per gram of hæmoglobin at N.T.P. This corresponds to two atoms of oxygen to each atom of iron. The compound formed, **oxyhæmoglobin,** is therefore conveniently expressed as $Hb.O_2$. This compound readily dissociates, giving off its oxygen again when the oxygen tension of the solvent is diminished (see Chapter XXVI.), all the oxygen taken up being removed by exposing the solution to a vacuum. The deoxygenated compound, to avoid ambiguity, is called **reduced hæmoglobin,**

with the symbol Hb. It can also be obtained from oxyhæmoglobin by adding reducing agents, *e.g.*, sodium hydrosulphite ($Na_2S_2O_4$), ammonium sulphide, ferrous salts. Oxyhæmoglobin solution is bright red in colour, reduced hæmoglobin reddish-purple. Arterial blood contains mainly $Hb.O_2$, venous blood is a mixture of $Hb.O_2$ with Hb.

Oxyhæmoglobin holds its oxygen so loosely that it can be easily displaced by other gases which form more stable compounds. Carbon monoxide is of most practical interest in this respect. Bubbling CO through a solution of oxyhæmoglobin results in the formation of **carboxyhæmoglobin,** Hb.CO, and evolution of the oxygen displaced. Carboxyhæmoglobin gives a characteristic red solution quite distinct from oxyhæmoglobin and usually described as cherry red. This compound is more stable than oxyhæmoglobin. The CO is not displaced by reducing agents, but it can be displaced by bubbling O_2 through the solution for some time, or by exposure to N_2 or H_2. The affinity of hæmoglobin for CO is 250 times greater than for O_2. This means that, if blood is exposed to air containing 1 part of CO to every 250 parts of O_2, it will take up approximately equal amounts of the two gases. Even small amounts of CO, therefore, will appreciably diminish the amount of oxygen which can be carried by the arterial blood. This is why it is so dangerous to inhale even small concentrations of carbon monoxide (*e.g.*, from coal gas or motor exhaust gas).

Nitric oxide forms an even more stable compound, **nitric oxide hæmoglobin.** Hydrogen sulphide hæmoglobin, **sulphæmoglobin,** is also a stable compound formed *in vitro* by passing H_2S through a solution of hæmoglobin. Its solution is dirty green in colour. It is not certain that the poisonous action of H_2S is due to the formation of this compound. It is found in the blood in a rare condition known as sulphæmoglobinæmia. Hydrocyanic acid hæmoglobin, **cyanhæmoglobin,** is not formed directly from oxyhæmoglobin and HCN, and is not the cause of the poisonous action of HCN. It is formed from methæmoglobin and HCN.

Oxidation. When blood or a hæmoglobin solution is exposed to air and light for a long time a brown pigment called **methæmoglobin** is formed. The same compound is obtained more readily by treating laked blood or hæmoglobin with mild oxidising agents such as potassium ferricyanide, ozone, nitrites, chlorates, which may also cause the appearance of methæmoglobin

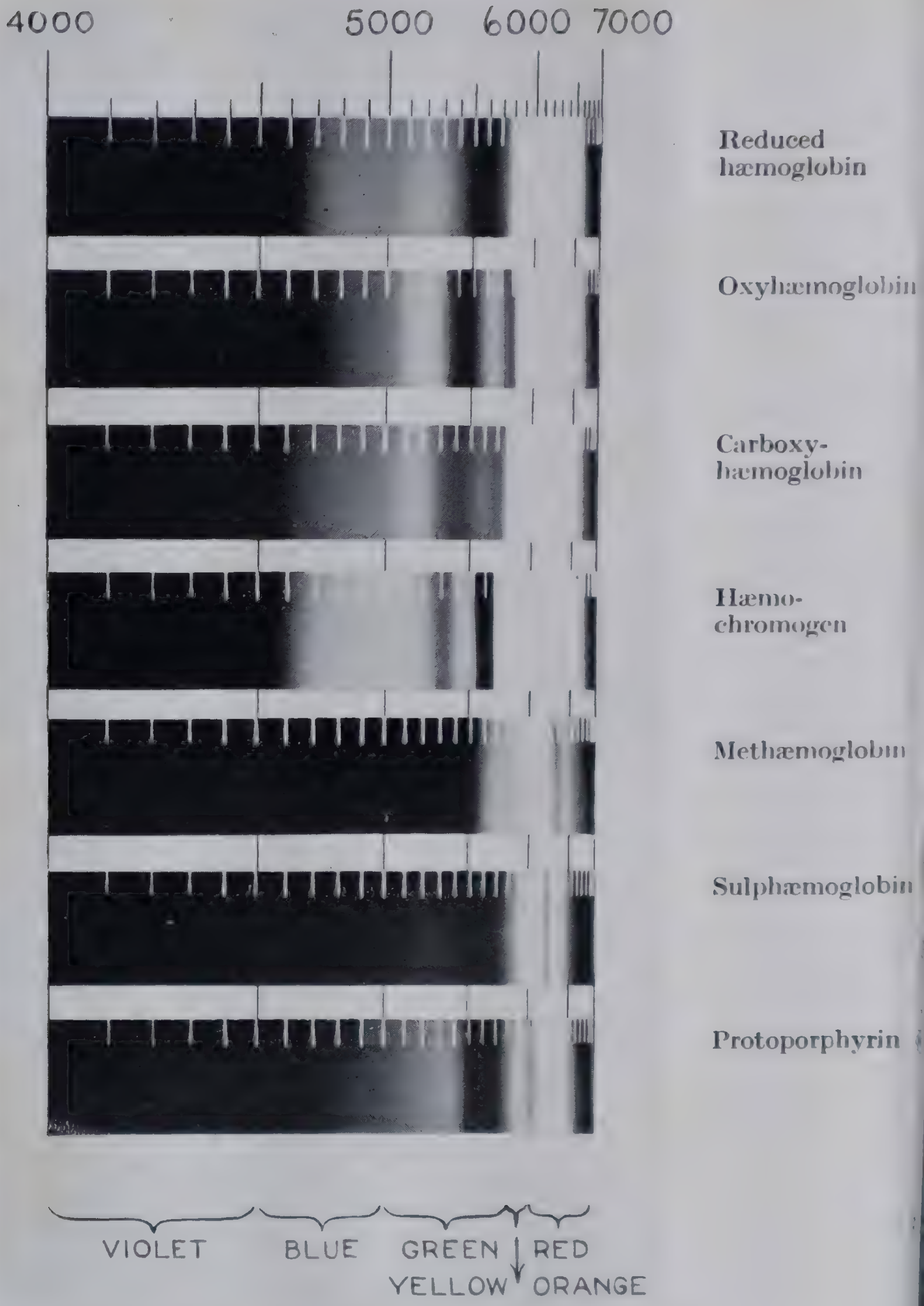


FIG. 12. Absorption spectra of hæmoglobin and its derivatives.

[To face p. 167

in vivo if administered. Methæmoglobin is a stable oxidation product of hæmoglobin and does not lose its oxygen in a vacuum. It is quite distinct from oxyhæmoglobin, *e.g.*, it forms a compound with HCN; oxyhæmoglobin does not. It can be reduced to reduced hæmoglobin by suitable reducing agents, *e.g.*, $Na_2S_2O_4$. Methæmoglobin contains only half as much added oxygen as oxyhæmoglobin, the iron carrying one oxygen atom instead of two. It is formulated either as HbO or HbOH. *Oxyhæmoglobin holds the oxygen loosely bound and is not an oxidation product of hæmoglobin; during oxidation of oxyhæmoglobin all this oxygen is evolved as molecular oxygen*, as expressed in the equation :—

$$\mathrm{Hb.O_2 + 2K_3Fe\,(CN)_6 + 2KOH \rightarrow HbO + 2K_4Fe\,(CN)_6 + H_2O + O_2}$$

$$\text{or} \qquad \mathrm{Hb.O_2 + K_3Fe\,(CN)_6 + KOH \rightarrow HbOH + K_4Fe\,(CN)_6 + O_2}.$$

(This is the basis of Haldane's method for estimation of oxygen combined with blood.) The iron in oxyhæmoglobin may be regarded as being in the *ferro* (divalent) state and in methæmoglobin in the *ferri* (trivalent) state. (In neither form is the iron ionisable.)

Absorption Spectra. These derivatives of hæmoglobin can be readily distinguished in solution by means of their absorption spectra. When white light passes through a solution of certain dyes, light of particular wavelengths is absorbed, so that if the light after passage through the dye is examined in a spectroscope, dark bands will be seen corresponding to the wavelength of light absorbed. The bands are not sharp like the Fraunhofer lines in the solar spectrum, but relatively broad with edges gradually fusing into the spectrum. It is customary to define the positions of these bands by the centre (and darkest) part of the band. The concentration of the solution and the thickness of the layer examined will determine the amount of light absorbed, *i.e.*, the width of the absorption band; with a very concentrated solution or thick layer the whole spectrum may be obliterated. At a suitable concentration (0·25% or blood diluted 1 in 60, examined in a ½-in. layer) hæmoglobin and its derivatives give very characteristic absorption bands, both in the visible and ultra-violet regions. The bands between red and green in the visible region are those commonly measured and used for the identification of blood pigments. Some typical spectra are shown in Fig. 12 and the positions of centres of the bands in the table on p. 168.

Absorption Spectra of Hæmoglobin Derivatives

Wave-lengths of Centres of Chief Bands in mμ

Reduced Hæmoglobin	565
Oxyhæmoglobin	578, 540
Carboxyhæmoglobin	572, 535
Methæmoglobin	630, (578), (540)
Sulphæmoglobin	618, (578), (540)
Hæmochromogen	558, 526
Protoporphyrin (acid)	600, 554
(Sodium D Lines)	590, 589

The bands of oxyhæmoglobin and carboxyhæmoglobin are very similar; they can, in fact, only be distinguished by careful measurement. The presence of carboxyhæmoglobin in oxyhæmoglobin cannot be detected in an ordinary spectroscope. This can only be done spectroscopically by an instrument devised by Hartridge in which two identical spectra are obtained, one above the other, but reversed so that the red end of one is over the violet of the other; by means of a micrometer screw one spectrum may be moved parallel to the long axis so that selected bands of the two spectra may be brought into line. This instrument is known as the **Reversion Spectroscope.** The presence of carboxyhæmoglobin in oxyhæmoglobin could be detected as follows :—

A solution of oxyhæmoglobin of suitable dilution is observed in the reversion spectroscope and the micrometer screw turned so that the α bands (those nearest the red end) coincide as in Fig. 13.

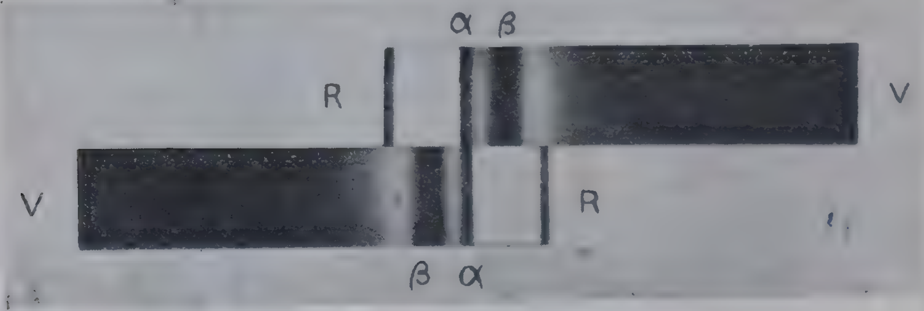


Fig. 13.

Without altering the setting, the oxyhæmoglobin is replaced by a similar dilution of the suspected blood. If carboxyhæmoglobin is absent, the appearance of the spectra will be unchanged as in Fig. 13 ;

if carboxyhæmoglobin is present, a slight shift of the bands towards the violet will cause the spectra to appear as in Fig. 14.

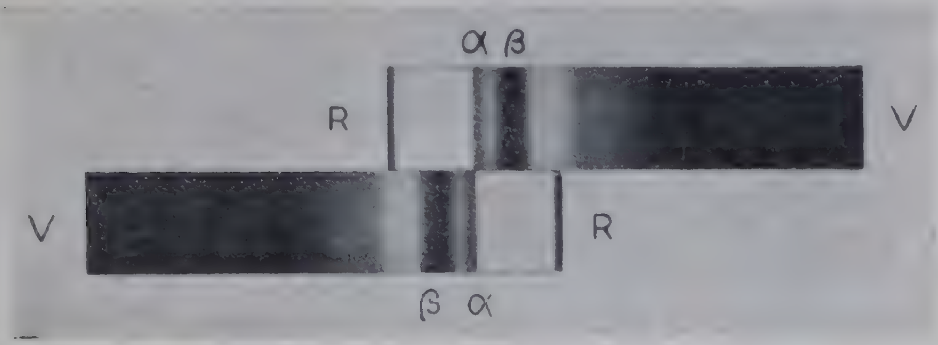


Fig. 14.

By comparison with suitable standards, the method can be used for the quantitative estimation of CO in blood.

Myohæmoglobin (*Myoglobin*, *Muscle Hæmoglobin*, *Myochrome*). The red pigment in striated muscle fibres differs from blood hæmoglobin in its absorption spectrum, the globin and the rate of combination with gases. The prosthetic group is the same. It has a smaller affinity for carbon monoxide. (See also p. 330.)

Breakdown of Hæmoglobin *in vitro*

Hæmoglobin only exists in the region of pH 7 at temperatures below 70° C. If treated in air with weak acid or alkali, or heated above 70° C., it breaks up into the pigment *hæmatin* and the protein *globin.*

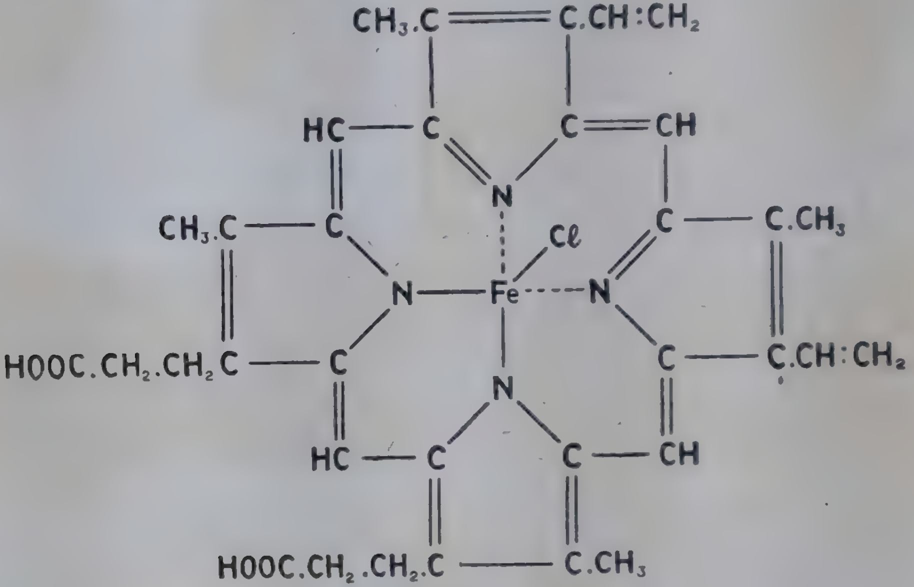
Globin is a strongly basic protein resembling the histones in general properties, but is an atypical member of this group in that the predominating basic amino-acids are histidine (8%) and lysine (9%), and not arginine (4%) (p. 106). Globin is very easily denatured and is only obtained undenatured by taking special precautions. Globin forms about 95% of the total hæmoglobin molecule.

Hæmatin is insoluble in water but soluble in acid or alkali (*acid hæmatin* and *alkaline hæmatin*). If oxyhæmoglobin is heated with glacial acetic acid and NaCl, globin is split off, but the pigment portion is not hæmatin, but another compound, **hæmin**, which so readily forms characteristic brown crystals that this reaction is used as a test for blood (Teichmann's test, Fig. 15).

The relationship between hæmatin and hæmin is that of a base and its chloride. Hæmatin can be formed from hæmin by treatment with NaOH, and hæmin from hæmatin by HCl. Since hæmin is so readily crystallised and purified, this compound has been most extensively investigated. The action of alkali may be expressed :—

$$\underset{\text{Hæmin}}{C_{34}H_{32}O_4N_4FeCl} + NaOH \rightarrow \underset{\text{Hæmatin}}{C_{34}H_{32}O_4N_4FeOH} + NaCl$$

Its structure, confirmed by synthesis, is :—



HÆMIN

The molecule is essentially four substituted pyrrole nuclei joined into a ring by methinyl (=CH—) groups. The iron is held by the nitrogen atoms partly by extra valencies and is not ionisable. This iron atom is responsible for the oxygen-carrying properties of hæmoglobin, although in hæmin it is in the oxidised ferri state. We can get a compound with the iron in the ferro state as in hæmoglobin by treating hæmin with alkali in the presence of a reducing agent, which gives a compound known as **hæm.** This reduction of hæmatin can be represented :—

$$\underset{\text{Hæmatin}}{C_{34}H_{32}O_4N_4FeOH} \rightleftharpoons \underset{\text{Hæm}}{C_{34}H_{32}O_4N_4Fe}$$

Hæm has the iron in ferro form and, like hæmoglobin, has an affinity for oxygen and gases. It is insoluble in water but soluble in dilute alkali. One function of globin is to convert hæm into a

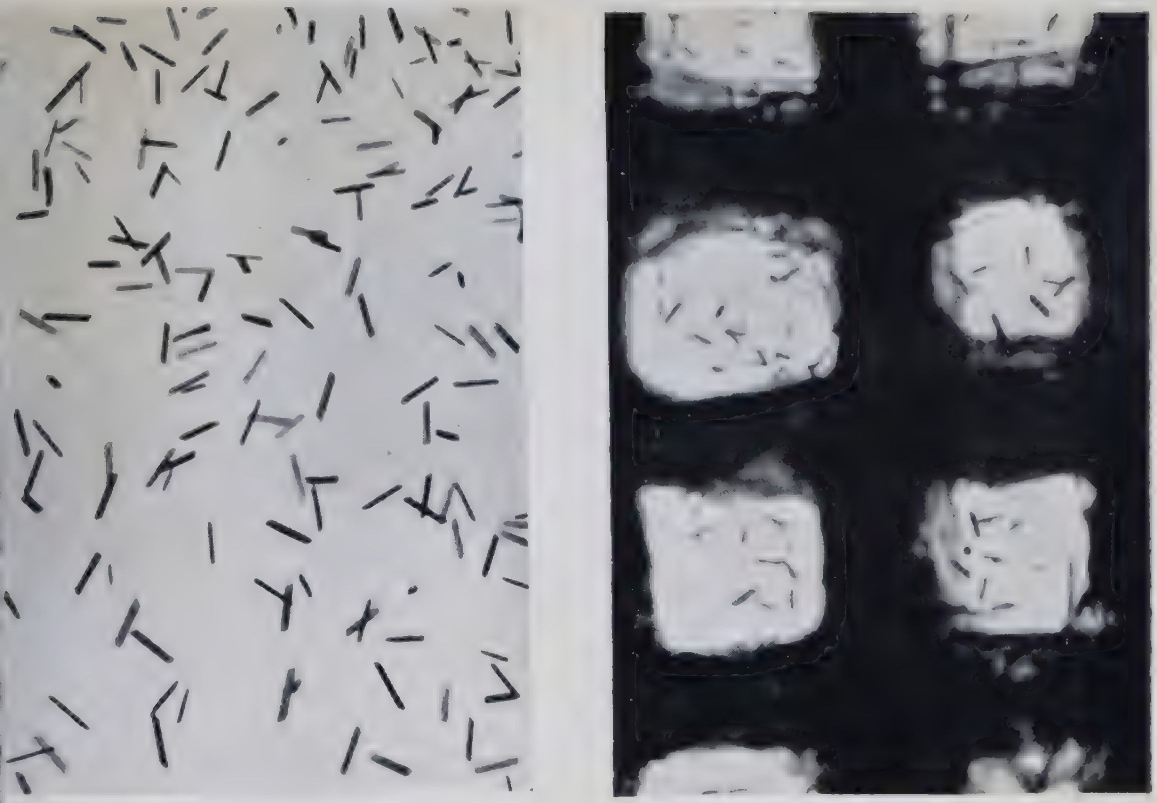


FIG. 15. Hæmin crystals.
(*a*) Formed from a blood film. × 145.
(*b*) Formed on blood-stained muslin. × 66.

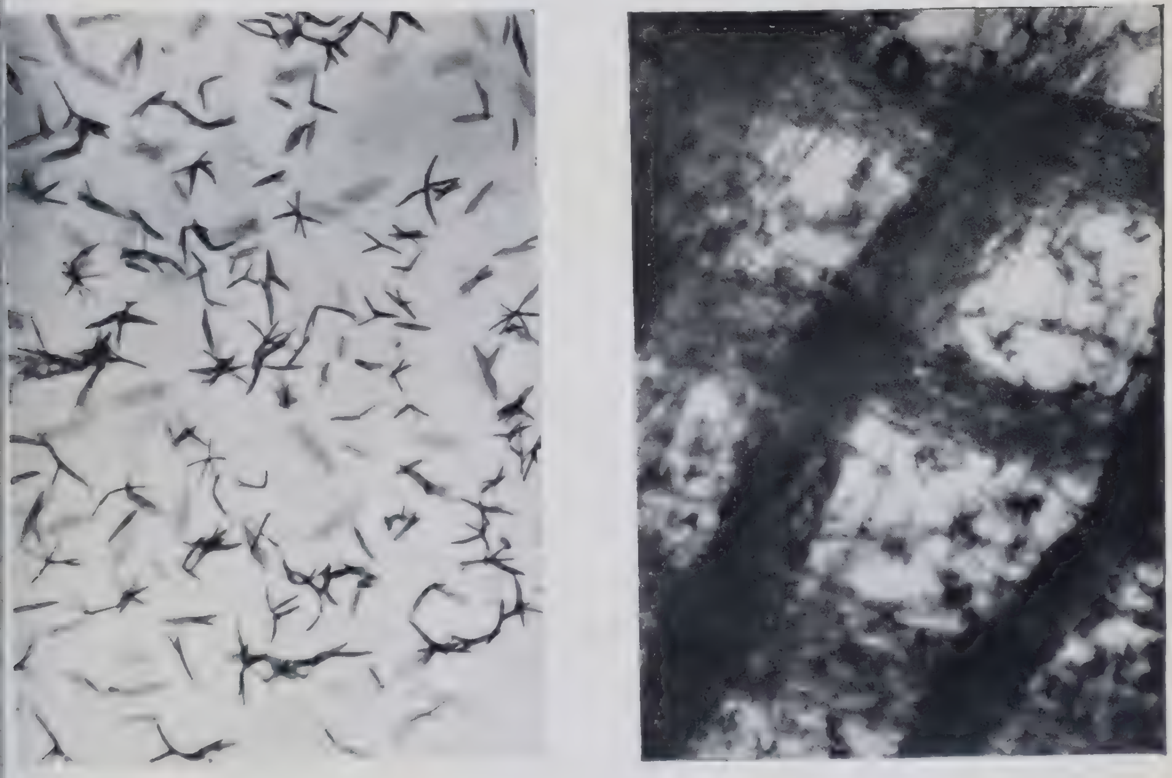


FIG. 16. Pyridine-hæmochromogen crystals.
(*a*) Crystals formed from a blood film. × 145.
(*b*) Crystals formed on blood-stained muslin. × 66.

very soluble compound suitable for the carriage of respiratory gases.

Hæm is not, however, formed, as might be expected, by treating hæmoglobin with alkali and a reducing agent. The compound formed in this way is called **hæmochromogen** and still contains globin ; it has a very characteristic spectrum, one band (λ 558) being especially sharp and visible at great dilution. The difference between reduced hæmoglobin and hæmochromogen lies in the globin, for the prosthetic group is hæm in each case. In hæmochromogen the globin is denatured, whereas the globin of hæmoglobin is native (*i.e.*, undenatured) globin. Hæmochromogen

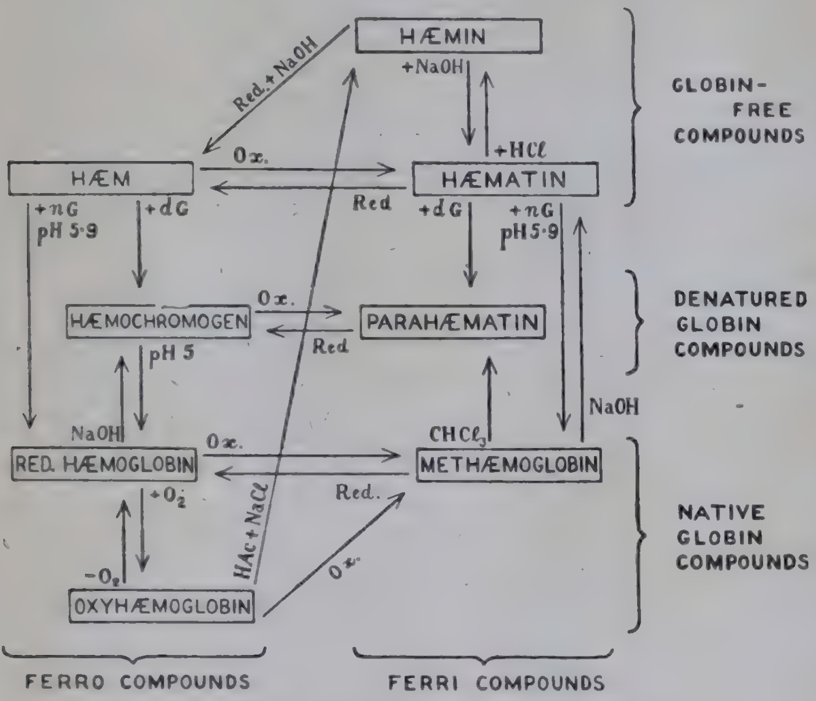

HÆMIN
+NaOH
+HCl
Red.+NaOH
HÆM
Ox.
Red
HÆMATIN
+nG
pH 5·9
+dG
+dG
+nG
pH 5·9
HÆMOCHROMOGEN
Ox.
Red
PARAHÆMATIN
pH 5
NaOH
NaOH
CHCl₃
RED. HÆMOGLOBIN
Ox.
Red.
METHÆMOGLOBIN
+O₂
−O₂
HAc+NaCl
Ox.
OXYHÆMOGLOBIN
GLOBIN-FREE COMPOUNDS
DENATURED GLOBIN COMPOUNDS
NATIVE GLOBIN COMPOUNDS
FERRO COMPOUNDS
FERRI COMPOUNDS

FIG. 17. Hæm derivatives containing iron.

dG, denatured globin ; *nG*, native globin ; *Ox*, oxidation ; and *Red.*, reduction.

Alternative names for some of the compounds in this diagram are :—

Hæm	*Hæmatin*	*Parahæmatin*
Reduced Hæm	Oxidised Hæm	Catahæmoglobin
Reduced Hæmatin	Oxyhæmatin	
Hæmochrome	Protohæmatin	

Strictly hæmochromogen should be called globinhæmochromogen and parahæmatin globinparahæmatin.

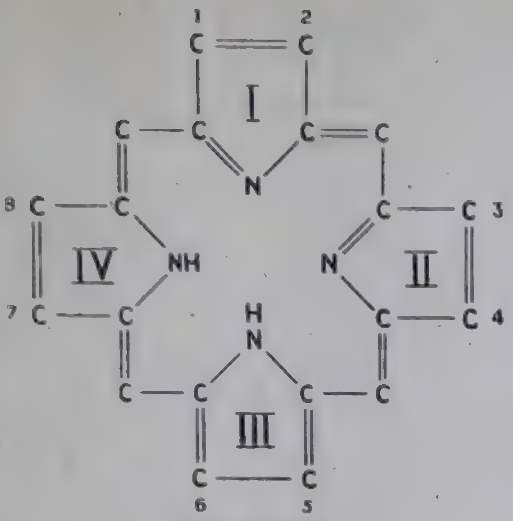
and hæmoglobin can be formed from hæm by the addition of the appropriate form of globin. In the same way oxidised hæm, that is, hæmatin, gives methæmoglobin on addition of native globin ; with denatured globin it gives a compound called **parahæmatin** or **catahæmoglobin.** Alternatively, methæmoglobin can be denatured directly to parahæmatin by treating with chloroform ; parahæmatin on reduction in alkaline solution yields hæmochromogen. The relationships between these compounds are shown in Fig. 17.

Hæmochromogens. Hæm readily combines with many nitrogenous bases besides globin. The compounds formed exhibit similar absorption spectra which are only distinguished by careful measurement of the bands. Among the bases with which hæm combines are albumin, ammonia, pyridine, piperidine, glycine and hydrazine. All these compounds are described as hæmochromogens, *e.g.*, ammonia hæmochromogen, pyridine hæmochromogen, so that the hæmochromogen formed from hæmoglobin is more accurately called globin hæmochromogen. The **pyridine hæmochromogen** formed from blood by treating with Takayama's reagent (glucose, pyridine, NaOH) crystallises with great readiness in salmon-pink needles, which are useful for the identification of blood in stains (Fig. 16).

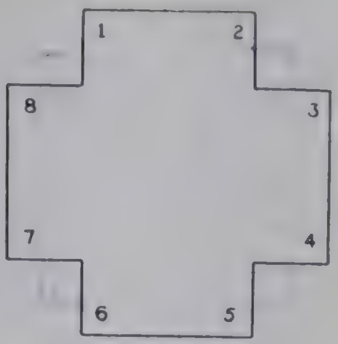
Cytochromes. Hæmochromogens appear to exist in almost all animal and plant tissues and, whatever the source, their spectra are almost the same ; the name *cytochrome* has been given to these naturally occurring hæmochromogens, replacing the older names *histohæmatin* and *myohæmatin*. The spectrum of cytochrome is the result of a mixture of the spectra of three hæmochromogens, cytochromes a', b', c'. Little is known of the nature of the nitrogen compounds combined with the hæm in these substances. The function of cytochrome in cellular respiration is described on p. 141.

Porphyrins (38, 88). So far all the hæm derivatives described have contained iron. This iron can be removed by the agency of strong acids without disrupting the arrangement of the pyrrole rings. The resulting compound is called a *porphyrin* ; hæm is a metallo-porphyrin, or more specifically, an iron porphyrin. Porphyrins are derivatives of a parent skeleton **porphin** (written in abbreviated form on the right) substituted in positions 1 to 8.

Porphyrins are widely distributed in nature, and many synthetic porphyrins are known. By treatment with a ferrous salt under



PORPHIN

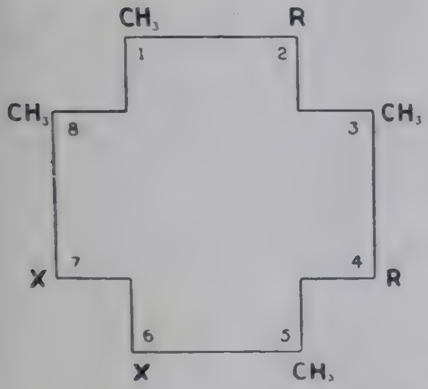


Abbreviated Form

appropriate conditions they yield iron porphyrins similar to hæm. Other metals can be introduced instead of iron.

The porphyrin corresponding to hæm is **protoporphyrin,** and is formed when hæmoglobin or hæmatin is treated with strong acids. If the vinyl ($—CH:CH_2$) groups take up H_2O, becoming $CHOH.CH_3$, **hæmatoporphyrin** is formed.

When hæmatoporphyrin is heated with soda lime an **ætioporphyrin** (ætioporphyrin III) is formed. This compound can be obtained from the **chlorophylls.*** These compounds and any of the porphyrins formed from hæmoglobin and chlorophyll can be represented by the formula :—



	R	X
HÆMATOPORPHYRIN	$-CHOH.CH_3$	$-CH_2.CH_2.COOH$
PROTOPORPHYRIN	$-CH:CH_2$	$-CH_2.CH_2.COOH$
ÆTIOPORPHYRIN	$-CH_2.CH_3$	$-CH_2.CH_3$

PORPHYRINS OF SERIES III

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* Chlorophyll *a* and *b* are closely related pigments composed of a magnesium porphyrin, of which two carboxyl groups in the side chains form esters, one with methyl alcohol and the other with an unsaturated alcohol, *phytol* ($C_{20}H_{39}OH$).

They are 1, 3, 5, 8-tetramethyl porphins or porphyrins of Series III.

Protoporphyrin III treated with a ferrous salt yields hæm, identical with that formed from hæmoglobin.

Porphyrins belonging to Series I with methyl groups in positions 1, 3, 5, 7 are also found in human tissues. In **coproporphyrin I** (**stercoporphyrin**) * and **uroporphyrin I** * the substituents in positions 2, 4, 6, 8 are the same, being $—CH_2 . CH_2 . COOH$ in the former and $—CH_2 . CH : (COOH)_2$ in the latter.

Coproporphyrin is present in normal fæces, serum and urine in minute amounts, which may be increased in disease. Uroporphyrin is the chief porphyrin excreted in congenital porphyrinuria and certain toxic conditions, *e.g.*, sulphonal poisoning. Coproporphyrin III has also been detected in cases of porphyrinuria. Hæmatoporphyrin is not excreted in urine, nor does it occur naturally. The term **hæmatoporphyrinuria** was used before methods of distinguishing porphyrins were available and should be avoided.

We shall see that the bile pigments can be regarded as porphyrins of Series III which have been broken at one of the methinyl (—CH=) linkages. Porphyrins are not, however, the biochemical intermediates between hæmoglobin and the bile pigments (see p. 177). They are, in fact, toxic and not formed in the *normal* breakdown of hæmoglobin.

Breakdown of Hæmoglobin *in vivo*

Red corpuscles do not remain in circulating blood for more than a few weeks, when they are destroyed by phagocytes, mainly in the reticulo-endothelial system of the spleen, bone marrow and liver, where the hæmoglobin is broken down, loses its iron and forms the bile pigment **biliverdin.** The iron is stored in the spleen, bone marrow and liver as brown granules of **hæmosiderin** (a pigment supposed to be mainly ferric hydroxide in organic combination), which is probably used for the formation of fresh hæmoglobin in the bone marrow. The biliverdin, along with another pigment, bilirubin, is excreted by the liver into the gall bladder. Amongst the evidence connecting the bile pigments and the breakdown of hæmoglobin there is :—

---

* Heating with soda lime would give an ætioporphyrin (ætioporphyrin I) isomeric, but not identical, with the ætioporphyrin III from hæmin. Other ætioporphyrins have been obtained synthetically, but not from natural products.

(1) The increased formation and urinary excretion of bile pigments under conditions involving the liberation of hæmoglobin, as by hæmolysing agents (p. 164), hæmolytic jaundice or injection of hæmoglobin.

(2) The formation of bile pigments at the site of a local bruise, *e.g.*, " black " eye.

(3) The occurrence of crystals of **hæmatoidin** (= biliverdin) in old blood clots after cerebral hæmorrhage.

We shall see that this is supported on chemical grounds. The *amount* of bile pigment formed, however, bears no constant relation to the amount of blood destruction, which suggests not only that hæmatin may be broken down to other products, but also that bile pigments may be formed from other sources such as myohæmoglobin or even chlorophyll derived from green vegetables. The chief function of the liver, which used to be regarded as the main seat of the formation of bile pigments, is probably their excretion. The formation of bile pigments can be demonstrated after extirpation of the liver.

The bilirubin formed in the reticulo-endothelial system is regarded by some as differing from the bilirubin of bile. The most noticeable difference is in the behaviour in the **van den Bergh test**, which consists in coupling the pigment with a diazo solution (*p*-sulphophenyldiazonium chloride) to form a red-violet solution. The bilirubin of bile gives an immediate (*direct*) reaction, whereas serum only gives a reaction after treatment with alcohol (*indirect* reaction). The chemical difference, if any, between the two pigments is not yet clear ; they are distinguished by the terms *hæmobilirubin* or indirect bilirubin, and *cholebilirubin* or direct bilirubin. Extensive study of the results of the van den Bergh test on pathological sera has led to further subdivisions of this reaction, a discussion of which is beyond our scope (Ref. 3). It must, however, be pointed out that there is *no chemical proof* of the existence of two bilirubins, and the possibility of the indirect reaction being a direct reaction inhibited by interfering substances removable by alcohol has yet to be eliminated.

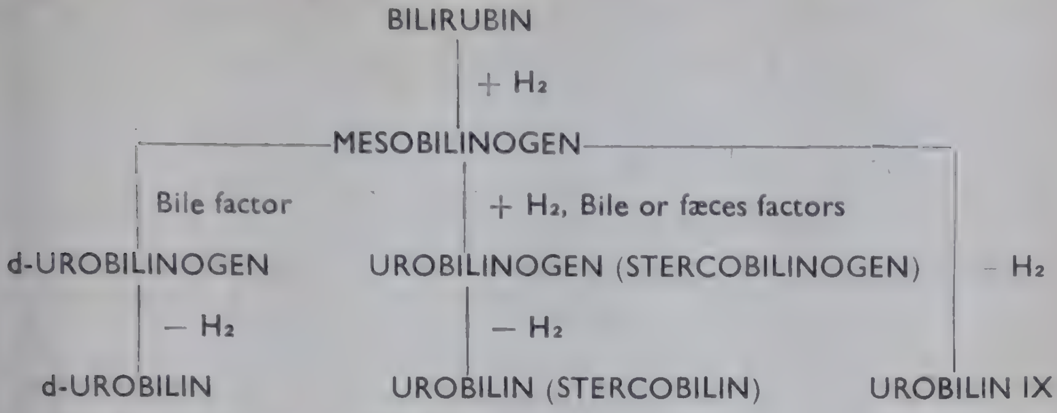
The amount of bilirubin in blood is normally very small (0·1–0·5 mg. per 100 c.c.). If the bilirubin content is abnormally high the pigment diffuses through the capillaries and gives the skin and mucous surfaces the characteristic yellow appearance of **jaundice.** This may be brought about by several causes, including excessive hæmolysis (physiologically in the *jaundice of the newborn* and pathologically in *hæmolytic jaundice*), hindrance of excretion due to injury of the polygonal cells of the liver, which excrete the pigment (*infective jaundice*), and prevention of excretion due to obstruction of the bile duct (*obstructive jaundice*). In the last condition bilirubin is excreted in the urine in appreciable amount.

In the gall bladder the colour of bile is mainly due to bilirubin and biliverdin, which are golden-yellow and green respectively. The former generally predominates in human bile, which is usually brownish-yellow. Green biles are more frequently encountered in herbivora.

After excretion from the gall bladder into the duodenum, biliverdin and bilirubin pass to the large intestine, where they are reduced by the action of bacteria to a colourless chromogen called **urobilinogen** or **stercobilinogen** which is partly converted into the brown pigment **stercobilin** (**hydrobilirubin**). Both are found in the fæces. Stercobilin, which is mainly responsible for the normal brown colour of fæces, is identical with the urinary pigment **urobilin.** The darkening of fæces on exposure to air is probably due to the conversion of urobilinogen into stercobilin.

There are three urobilins. The two chromogens, urobilinogen and **mesobilinogen** (**mesobilirubinogen**), were once thought to be identical. The latter, but not the former, has been isolated from pathological urine. On exposure to air and light mesobilinogen is converted into a brown pigment, but this pigment, **urobilin IX** α, is not identical with the urobilin isolated from urine or fæces. Urobilin is readily obtained in crystalline form even from urine and is lævorotatory, whereas urobilin IXα is optically inactive and is difficult to obtain, even from isolated mesobilinogen which has been exposed to air. There is, furthermore, a difference between the urobilin obtained from urine and that formed in bacterially infected bile. The latter is dextrorotatory and has been termed **d-urobilin.** It can be formed from mesobilinogen. It would seem that mesobilinogen is the primary product of the reduction of bilirubin by bacteria ; in the presence of a bile factor mesobilinogen is converted to **d-urobilinogen** but in the presence of bile and fæces factors urobilinogen (stercobilinogen) is formed. The three urobilins are formed from these chromogens by oxidation. The changes can be summarised in the scheme shown on p. 177.

In the absence of bile excretion, *e.g.*, due to obstruction of the bile duct, the fæces are pale or almost white. In the newborn, in which the alimentary canal is practically free from bacteria, the dark green meconium (fæces) contains biliverdin ; this pigment is largely responsible for the greenish colour of diarrhœic stools of infants.



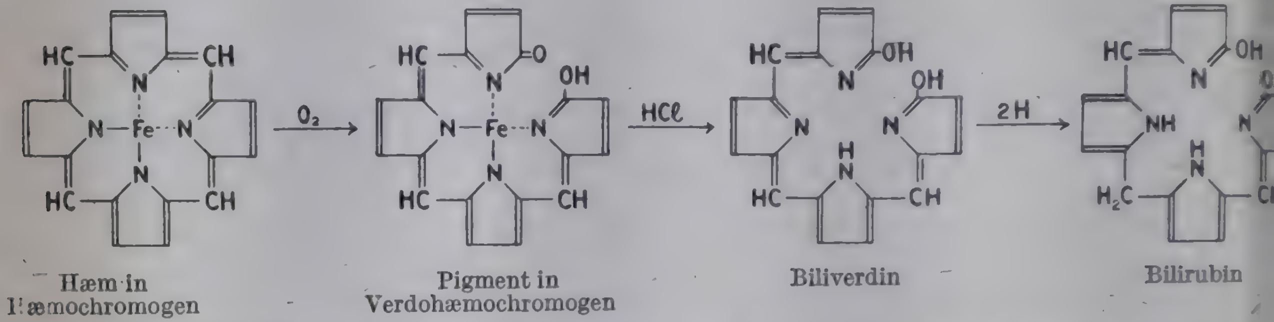
For formulæ, see p. 178-179.

Part of the bile pigments escapes excretion in fæces by being absorbed in the large intestine, probably as urobilinogen. This is partly excreted by the liver, either as such or after oxidation to bilirubin, thus completing a bilirubin cycle. A small amount escapes excretion by the liver and passes into the general circulation as urobilinogen, which is excreted by the kidney. When the urine is exposed to air, urobilinogen is oxidised to the pigment urobilin. Normally the amount of urobilin thus formed is so small that its colour is insignificant compared to the other urinary pigments. The amount is increased by conditions involving excessive hæmolysis (*e.g.*, hæmolytic jaundice, pernicious anæmia). A slight temporary urobilinuria may be observed in constipation, due to increased absorption of urobilinogen from the large intestine.

Chemistry of the Bile Pigments and their Formation (7, 38)

Until recently and in spite of the absence of any conclusive evidence, it was assumed that bilirubin was formed from either hæmatin or protoporphyrin liberated from the breakdown of hæmoglobin. It was further accepted that bilirubin was the primary bile pigment and biliverdin its oxidation product. It now seems that neither hæmatin nor protoporphyrin are normal products of hæmoglobin breakdown and that the primary bile pigment is biliverdin, from which bilirubin is formed by reduction. The probable intermediate in hæmoglobin breakdown is a hæmochromogen which is oxidised to a green **verdohæmochromogen.** This compound then loses iron and protein, giving biliverdin.

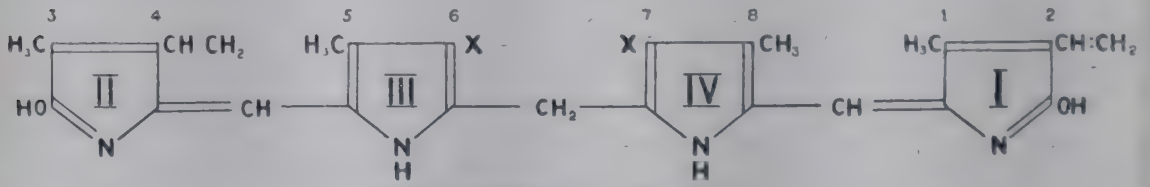
Lemberg has obtained biliverdin in this way from pyridine-hæmochromogen *in vitro*, and has produced evidence of the presence of verdohæmochromogens in cells and the possibility of such reactions. The change of hæm to bilirubin can be represented thus :—


HC
CH
N
N
Fe
N
N
HC
CH
O_2
HC
O
OH
N
N
Fe
N
N
HC
CH
HCl
HC
OH
OH
N
N
N
H
N
HC
CH
2H
HC
OH
N
NH
N
H
N
H_2C
Hæm in
Hæmochromogen
Pigment in
Verdohæmochromogen
Biliverdin
Bilirubin

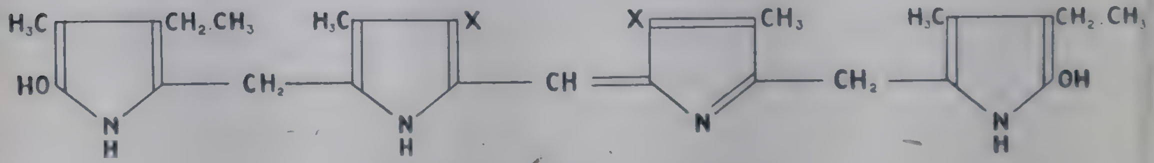
Substituents, which are the same throughout, have been omitted. Note that in verdohæmochromogen the porphyrin ring has opened (one carbon being lost), but iron is still held. This iron is more readily removed than from hæm.

It is more usual to write the formula of bilirubin in the straight form as in the scheme below which shows the relationship between bilirubin, urobilin IXα, mesobilinogen, urobilinogen and urobilin.

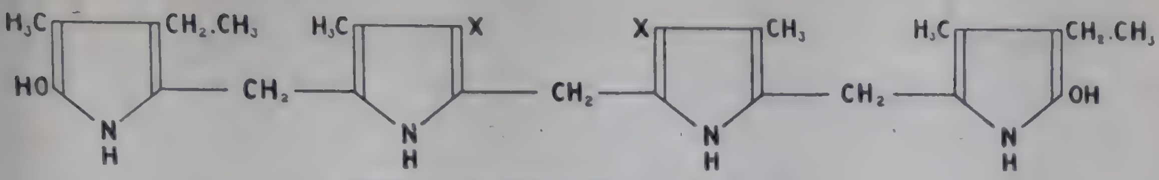
Oxidation of bilirubin gives biliverdin or another green pigment, **uteroverdin,** $C_{33}H_{34}O_6N_4$, found in dog's placenta. Further oxidation gives blue (**bilicyanin** or **cholecyanin**), red and yellow (**choletelin**) pigments, the colours of which are seen in Gmelin's test, in which bilirubin is oxidised with fuming nitric acid. Many of the colours are seen *in vivo* on the skin over a bruise, where there is a local breakdown of hæmoglobin in the injured tissue. For the probable structure of these pigments see Ref. 38.


3
4
5
6
7
8
1
2
H_3C
CH CH₂
H_3C
X
X
CH_3
H_3C
CH:CH₂
II
III
IV
I
HO
CH
CH_2
CH
OH
N
N
H
N
H
N

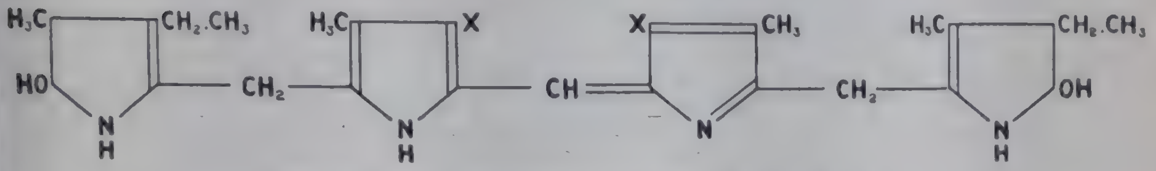
BILIRUBIN, $C_{33}H_{36}O_6N_4$


H_3C
$CH_2.CH_3$
H_3C
X
X
CH_3
H_3C
$CH_2.CH_3$
HO
CH_2
CH
CH_2
OH
N
H
N
H
N
N
H

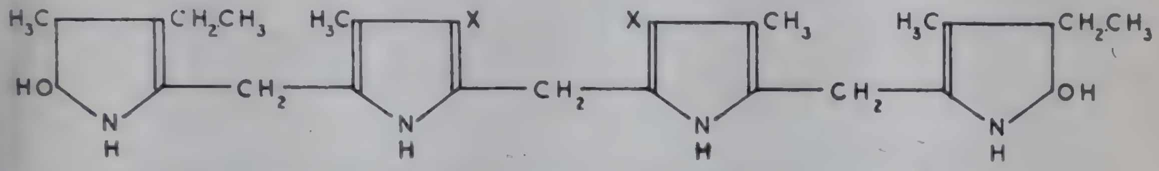
UROBILIN IXα, $C_{33}H_{42}O_6N_4$



MESOBILINOGEN, $C_{33}H_{44}O_6N_4$



UROBILIN (STERCOBILIN), $C_{33}H_{46}O_6N_4$



UROBILINOGEN (STERCOBILINOGEN) $C_{33}H_{48}O_6N_4$

X = $-CH_2 . CH_2 . COOH$ in each formula. The numbering on the formula of bilirubin shows the positions of the substituents in protoporphyrin. Note that rings II and I in urobilin and urobilinogen are not pyrrole, but the more saturated pyrroline rings which, owing to the substituents ($-CH_3$ and $-OH$ in II and $-CH_2 . CH_3$ and $-OH$ in I), have two asymmetric carbon atoms each. Hence the optical activity of urobilin.

BODY FLUIDS RESEMBLING PLASMA IN COMPOSITION

Lymph. In composition lymph (the fluid of the lymphatic vessels) can be regarded as a transudate of plasma through a membrane which is partly permeable to protein. The electrolyte and crystalloid content is approximately the same as that of plasma ; the protein content is approximately half that of plasma. The protein has a greater proportion of low molecular weight protein (albumin), and consequently a larger osmotic pressure *per gram* than plasma protein. The colloid osmotic pressure of lymph is lower than that of plasma. The protein content of lymph varies according to the part of the body from which it is collected ; it includes fibrinogen, since lymph usually clots on standing. After a meal containing fat, the intestinal lymph is " milky," containing a high percentage (5%–15%) of emulsified fat. In this condition it is called **chyle.**

Tissue Fluid, the fluid bathing the cells and occupying the intercellular spaces, is probably similar to lymph in composition. Tissue fluid forms the connecting link in the transport of nutrient material between the blood capillaries and the tissue cells.

Synovial Fluid is the clear viscid alkaline fluid in joint cavities, bursæ, etc., whose chief function is the lubrication of the rubbing surfaces of the moving joints. In composition it resembles plasma except for a lower protein concentration (about 5%). The albumin : globulin ratio is roughly the same as in plasma.* It contains a mucus-like substance, **synovin,** of unknown nature.

BODY FLUIDS RESEMBLING A PLASMA DIALYSATE IN COMPOSITION

Cerebrospinal Fluid (37). Normal cerebrospinal fluid is a clear colourless alkaline liquid, specific gravity 1·006–1·008, which contains a few lymphocytes (1–5 per cu. mm.). Its composition, so far as it is known, is nearly but not exactly that of an ultrafiltrate or dialysate of plasma. The calcium content corresponds with that of the diffusible calcium of plasma (5 mg. per 100 c.c.), but the sugar is rather lower (45–100 mg. per 100 c.c.) and the chloride higher (700–760 mg. NaCl per 100 c.c.). There is a small amount of protein (10–35 mg. per 100 c.c.). The urea content is about the same as that of plasma. The composition varies slightly with the site of withdrawal.

Intraocular Fluid. The composition of **aqueous humour** is very similar to that of cerebrospinal fluid without the cells. The most noticeable characteristic of the gel-like **vitreous humour** is that it contains a mucoprotein (0·02%) and another unidentified protein (0·02%) not found in aqueous humour, otherwise the composition of the two humours is very similar.

* Values determined on fluid from cases of arthritis with joint effusion (Carjori and Pemberton).

CHAPTER XIII

THE COMPOSITION OF THE TISSUES (1, 4, 5)

It may at first seem surprising that we have little accurate knowledge of the composition of human tissues. We know that a number of constituents can be obtained from mammalian tissues, most of which have been detected in human tissues; but there has been little attempt to correlate the quantitative distribution of characteristic substances like coagulable protein, nucleoprotein, collagen, phospholipides and fat with the histological structure of the tissues. Our detailed quantitative information is confined to low molecular weight substances like salts, glucose and creatine, which could not be expected to appear in the histological picture. Fresh human tissue suitable for analysis is, of course, difficult to obtain; but even animal tissues do not seem to have been extensively analysed as a whole, probably on account of the extremely laborious nature of the task. For lack of suitable analyses of human tissues, the table below, giving the percentage composition of the major groups in ox

Composition of Ox Tissues

(*Values from Ref.* 65 *and Other Sources*)

	Water	Protein	Lipides	Glycogen	Ash	Collagen
Blood	80·8	18·1	0·2	—	0·9	—
Brain	80·6	8·8	9·3	—	1·1	—
Fat (retroperitoneal)	4·3	—	95·0	—	0·2	—
Heart	62·6	18·0	20·4	—	1·0	—
Kidney	76·7	16·9	4·8	0·4	1·2	—
Ligamentum nuchæ	57·6	40·0*	1·1	—	0·5	7·2
Liver	71·2	20·7	4·5	1·5	1·6	—
Lung	79·7	16·1	3·2	—	1·0	—
Muscle	75·9	18·4*	0·9	1·5	1·3	1·7
Tendon	62·9	34·7*	1·0	—	0·5	31·6

* Including collagen.

tissues, is presented. Even amongst herbivorous animals there is considerable variation, *e.g.*, the lipides of ox liver are 4·5%, compared with 9% in the sheep (human 4·3%), whereas ox heart is 20%, compared with 12% in sheep and 7% in man. The values in the table must, therefore, be taken as a very rough approximation to those of human tissues.

A few tissues which have constituents of particular interest have been examined in greater detail. Of these, blood, bones and teeth are described elsewhere (Chapters XII and XXIII).

Connective Tissues

The component parts of connective tissues proper are essentially white collagen fibres, yellow elastic fibres, ground substance or matrix, fat and cells. Characteristic substances are found in the first four. It is more convenient to describe these than individual tissues, which are made up of varying proportions of the component parts.

White Collagen Fibres. The principal constituent is the insoluble protein (or type of protein) **collagen.** This protein is characterised by forming **gelatin** (p. 108) when it is boiled with water for a few hours. Collagen and gelatin are practically free from cystine, tryptophan and tyrosine, and are rich in glycine and the prolines. Collagen, although insoluble, is attacked in the stomach, giving gelatin which is easily digested.

Yellow Elastic Fibres. The characteristic substance is the insoluble protein **elastin,** which resembles collagen in composition but does not give gelatin on boiling with water. It is not digested in the alimentary canal.

Ground Substance. The principal constituents are two proteins, **chondromucoid** and **chondroalbumoid** (chondroalbuminoid). The former, on hydrolysis, yields **chondroitin sulphuric acid** (p. 109) and is a typical mucoid. The mucoids of different connective tissues are probably not identical, although all contain chondroitin; they are called after the tissue, *e.g.*, tendomucoid, osseomucoid. Chondroalbumoid, which is only present in small amount, somewhat resembles elastin, but is digested by gastric juice.

Fat. The fat of connective tissues is predominantly neutral fat. The nature varies according to the locality, *e.g.*, retroperitoneal fat is more saturated and " harder " than bone marrow fat. The fat cells are bounded by a protein membrane, so that analysis would give mainly fat, water and a little protein. The fat is coloured by lipochromes (p. 120).

These are the characteristic substances of connective tissues. The composition of an actual tissue depends upon the relative proportions of the components. The table gives some typical analyses, mostly by Gies.

	White Fibrous Tissue (Ox Tendon)	Yellow Elastic Tissue (Ligamentum nuchæ)	Costal Cartilage	Bone with Marrow (Dog's Femur)	Fat (Retro-peritoneal)
Water . .	62·9	57·6	67·7	19·1	4·3
Inorganic .	0·5	0·5	2·2	38·2	0·19
Organic . .	36·6	41·9	30·1	41·6	95·7
Lipides .	1·0	1·1	—	25·6	(mostly fat)
Coag. Protein	0·2	0·6			
Mucoid .	1·3	0·5			
Elastin .	1·6	31·7			
Collagen .	31·6	7·2			
Extractives .	0·9	0·8			

Muscular Tissues

Skeletal Muscle. The gross composition of voluntary muscle is approximately 75% water, 20% protein and 5% lipides, carbohydrates, inorganic salts and extractives.

The Proteins. A small part of the total protein of muscle is derived from the connective tissue (collagen) and nuclei (nucleoprotein); the remainder (about 18% of the muscle) consists of four proteins, **myosin, myogen, globulin** *X* and **myoalbumin.** The sarcolemma is said to be composed of an elastin-like protein.

Myogen is soluble in water and behaves like an albumin. It is very readily denatured at its isoelectric point (about pH 6·3).

Myosin behaves as a globulin (hence the name muscle globulin) and is insoluble in water, but soluble in salt solutions. Its isoelectric point varies with the salt concentration; values of pH 5·1–5·5 and pH 6·2–6·6 have been given. Myosin solutions very readily form firm gels at 40° C. or on dilution, and are noticeably viscous. X-ray studies have shown that myosin, which forms more than half of the muscle protein, is almost certainly the active elastic principle of muscle. The polypeptide chains of myosin lie along the axis of the muscle fibril. In the relaxed muscle the chains are folded in an α form like α-keratin (p. 97). On stretching the chains can be extended to a β form like β-keratin. When the fibrils contract, the myosin molecules contract to a length shorter than the α form by a further folding of the polypeptide chain to a " supercontracted " form. Keratin only " supercontracts " after chemical treatment which attacks the disulphide linkages by which the polypeptide chains in keratin are thought to be held together.

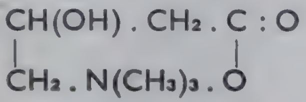
Myogen and myosin can be extracted from minced fresh muscle (washed free from blood) by salt solutions. (After rigor has set in only a small part of the protein is extractable.) If the fresh muscle is minced with ice and a little salt and filtered in a press a viscous fluid, formerly called " *muscle plasma*," is obtained. (It is a strong solution of the proteins in salt solution.) This fluid forms a gel (" clots ") on standing at room temperature or 37° C. The " clotting " has been ascribed to the conversion of " myosinogen " and " paramyosinogen " to insoluble myosin, analogous to the formation of fibrin from fibrinogen. The simpler conception of gel formation is preferable until the existence of myosinogen can be satisfactorily established.

Lipides (fats, lecithin and cholesterol) are only present in small amount (about 0·2%).

Carbohydrates. Glycogen is usually between 0·5% and 1·0%, and hexosemonophosphate about 0·05%.

Inorganic Salts. The ash of muscle amounts to 1–1·5%. This is predominantly potassium phosphate. Approximate values for the chief inorganic elements in fresh muscle are K 0·3%, Na 0·06%, Mg 0·02%, Ca 0·007%, Cl 0·04%, P 0·2%. There are traces of sulphate. Most of the phosphorus is organically bound as creatine, adenosine and hexose phosphates in the resting muscle.

Extractives. Under this heading are included some simple substances, other than those already mentioned, which can be extracted from muscle by water or alcohol. Most of these are described in greater detail elsewhere. Amongst the non-nitrogenous are **lactic acid** (*sarcolactic acid*), 0·015% in resting well-oxygenated muscle, and **inositol,** formerly called "muscle sugar," although neither a carbohydrate nor peculiar to muscle. The nitrogenous extractives include **creatine** (0·35%), **adenylic acid** (0·15%) with small amounts of inosinic acid, hypoxanthine and xanthine probably derived from it, **carnosine** (0·3%) and **carnitine** (0·1%). The last two are peculiar to muscle, although their function is unknown. Carnosine (β-alanylhistidine) is not present in all species ; in some animals it is replaced wholly or in part by its N methyl derivative, **anserine.** Human muscle contains carnosine, but does not appear to have been examined for anserine. Carnitine is the betaine of β-hydroxybutyric acid :



CARNITINE

Pigments. The pigment of red muscle is **myohæmoglobin** (*myoglobin, myochrome*), closely resembling hæmoglobin. **Cytochrome** (*myohæmatin*) is also present.

Heart Muscle. The creatine and carnosine contents of heart muscle are lower than those of skeletal muscle, and the phosphatide and cholesterol contents higher. Its metabolism differs from that of skeletal muscle in that lactic acid is utilised more than is glucose (p. 233).

Plain Muscle. The nucleoprotein content of plain muscle is higher than that of skeletal muscle, as would be expected from the structure of the muscle cells. Only very small amounts of creatine and carnosine are present. The ratio Na : K is about 1 : 2 instead of 1 : 5, as in skeletal muscle. The lipide, glycogen and lactic acid contents are about the same.

Nervous Tissue

The chief characteristic of nervous tissue is the high proportion of compound lipides and cholesterol. The water content of different nervous tissues varies from 83% to 85% (fœtus 92%) in the grey matter of the brain to 60% in peripheral nerves. According to Koch, the chief constituents of the white and grey matter of brain are :—

	White	Grey
Water	70%	84%
Protein	10%	8%
Phosphatides	8·5%	3·7%
Cerebrosides	5%	3%
Cholesterol	5%	0·7%
Inorganic	0·8%	0·8%

The proteins include a globulin (or globulins), nucleoprotein, and a **neurokeratin.** Lecithin forms the greater part of the phosphatides, kephalin is present in fair amount, but sphingomyelin only in small amount. The chief cerebrosides are phrenosin and kerasin ; the others are present in small amount. Cholesterol is present in the free state, about 1·9% in whole adult brain (less in infants). The fatty acids of brain have a higher average molecular weight than those of other tissues and are, on the average, less saturated. There is relatively little neutral fat. Potassium is the most abundant metallic ion.

Amongst the other constituents of brain are creatine, lactic acid, hexosephosphates, methylglyoxal, glycogen and inositol.

Other nervous tissues are similar in composition.

Skin and Appendages

The principal layers of the skin are the epidermis and dermis (corium). The latter is a dense connective tissue which carries the blood vessels and nerves supplying the epidermis. It consists mainly of collagen fibres with some elastin from the elastic fibres present.

The epidermis consists of many layers of cells ; those on the outer surface, being dead, form the "horny" layer of the skin, in which the characteristic protein of the epidermis, **keratin**, is laid down.

Keratin is an insoluble scleroprotein characterised by a high sulphur, liberal tyrosine and poor proline content in contrast to collagen. It does not yield gelatin on boiling and is indigestible. The composition of keratins varies slightly in different species.

The lipides of human epidermis (about 7%) contain a much greater proportion of phospholipides and cholesterol than does subcutaneous fat. About one-tenth is free cholesterol ; associated with this are traces of a sterol which is converted to vitamin D on exposure of the skin to sunlight (p. 356).

The other constituents of skin include sugar (about 60 mg. per 100 g.), glycogen, mucin, Na, K, Ca, Mg and Cl. In man there is more Na than K.

The pigments of skin are **melanins**, and are increased in white races on exposure of the skin to sun ; when present in large amount they give the black appearance typical of the skin of negroes. Lipochromes are also present.

The sebaceous glands produce a waxy secretion, *sebum*, consisting of a mixture of lipides, including cholesterol esters. For sweat see p. 463.

Hair. The chief constituent of hair is keratin.* That of human hair is so rich in cystine (15·6–21·2%) that human hair may be easily differentiated from all other animal hair or wool. The sulphur of human hair is almost entirely in the form of cystine. According to Hawk, red hair has the highest sulphur content. Other constituents of hair are water (12–15%), lipides (3·4–5·8%) ; the ash is from 1% to 1·5%, of which about one-tenth is silica.

The colour of hair can be ascribed to three causes. Blonde, brown and black is due to varying amounts of melanin ; bright red human hair contains a pigment with a high content of iron ; grey is caused by an absence of pigments and white by an increased proportion of calcium carbonate and phosphate (36% of the ash instead of 14–20% in coloured hairs)—the total ash is not increased.

Nails. Human nails are similar in composition to animal horns and hooves. The chief constituent is keratin, but not the cystine rich keratin of the hair ; its cystine content is about 5%.

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* For the structure of hair keratin see p. 97.

PART II

CHAPTER XIV

THE BIOCHEMISTRY OF DIGESTION (1, 5)

THE ultimate end of digestion is mainly the conversion of colloidal and often insoluble substances of high molecular weight into simple soluble substances, which are either diffusible or capable of forming diffusible complexes. These changes take place in the alimentary canal with very small expenditure of energy under the influence of hydrolytic enzymes. In this way starch is converted into glucose and proteins into amino-acids. Both these end products are readily absorbed into the blood. Fats are broken down to glycerol, which is readily diffusible, and fatty acids, which are not. The latter, however, form diffusible complexes by combination with bile salts.

Advantages of Digestion. While the conversion of food into soluble diffusible substances is the main object of digestion, it is by no means the only end attained. The composition of the body proteins, fats and polysaccharide is not the same as that of the proteins, fats and polysaccharides of food. Only the units of which complex substances are formed are common to both the body and the food. For conversion of food protein into tissue protein it is essential that the former be broken down to the amino-acids from which the characteristic tissue protein can be constructed. In the same way starch is converted into glucose, so that glycogen, the characteristic polysaccharide of animals. may be formed. The modification of food fat, when resynthesised from glycerol and fatty acid in the body, is not so great as in the other two groups, and the fat in the body may be influenced in some degree by the nature of the ingested fat.

Digestion is of value in economising simple substances such as the disaccharides. Disaccharides are diffusible and could be readily absorbed if not digested, but absorbed disaccharides are not utilised in the body and are excreted as foreign substances practically unused. By digestion to hexoses these unusable substances are converted into valuable food.

Another important function of digestion is to counteract the toxicity of proteins. Foreign proteins injected into the blood stream of an animal are usually very toxic. This specific toxicity of proteins is destroyed when the protein is hydrolysed to amino-acids.

While the process of digestion destroys the toxicity of proteins, it produces toxic substances from fat, for large amounts of fatty acids introduced into the blood are toxic, but this toxicity of fatty acids is obviated by immediate resynthesis of fatty acids to fat after passing into the villi.

A further benefit might be claimed from digestion in that it prevents the too rapid absorption of food, and so enables the blood to distribute the absorbed substances without undesirable effects, due to overloading of the transport mechanism. If all our food were in a form which could be rapidly absorbed, we should probably be obliged to increase the customary number of meals per day. The power of digestion also enormously increases the possibilities of variation in our diet and our consequent enjoyment, a fact probably too well realised by those whose digestive mechanisms have become impaired.

The Enzymes of Digestion. The enzymes concerned in the breakdown of various foodstuffs are described in detail when the utilisation of the particular group of foodstuffs is considered. These enzymes are more than adequate for the complete digestion of the food by the time it gets to the lower part of the small intestine. It will be seen that in most instances a succession of enzymes is provided for each group of substances during passage down the alimentary canal ; the enzymes overlap in their action, so that a substance may escape the action of one enzyme and yet be fully digested. There is a very generous provision of enzymes, for portions of the alimentary canal may be removed without serious impairment of digestive function. Further, there is evidence that the amounts of enzymes secreted may be controlled by the dietetic habits of the individuals. A powerful secretion of an enzyme, previously little employed, may be gradually induced by continuous ingestion of the substance which the enzyme attacks ; conversely, elimination of a substance from the diet will diminish the secretion of the enzyme which digests it.

THE DIGESTIVE JUICES

It is assumed that the reader is acquainted with the physiological aspects of digestion—the stimuli promoting secretion of the digestive juices and the movements of the alimentary canal in response to food. Here we must confine our attention to the chemical nature of the digestive juices.

Saliva

Being a mixture of the secretions of three pairs of glands, saliva shows considerable quantitative variation in composition. The secretions of the three glands differ; the submaxillary secretes most of the protein mucin, while the parotid secretion contains practically none. The amount of secretion from each gland varies with the stimulus and nature of the food. There is, as might be expected, considerable variation in the figures given for the amount of saliva secreted per day. It is usually stated to be between 1,000 and 1,500 c.c. The reaction of saliva is also variable, and values from pH 5·8 to 7·6 have been recorded. It can be taken as most commonly lying between pH 6·4 and 7·1, the secretion usually being slightly acid rather than alkaline, but varying throughout the day. The composition of saliva, of course, varies according to the stimulus evoking its secretion, so that the total solids may vary from 0·3% up to 1·4%. A saliva of 0·6% total solids would contain about 0·4% organic and 0·2% inorganic material. The chief organic constituent is mucin (over 0·3%), and this is the predominant component of the more concentrated salivas. The other organic constituents include small amounts of albumin and globulin, ptyalin, urea and uric acid, and traces of thiocyanic acid.

The amounts of the inorganic constituents vary considerably, even in the same individual. The following figures (mg. per 100 c.c.) will serve as an example: K 38, Na 26, Ca 8, Mg 1, HCO_3 60, Cl 50, PO_4 10.

The functions of saliva, apart from providing an amylase, are chiefly moistening dry food and assisting deglutition by the lubricating quality of mucin.

The mucin of saliva is precipitated in long strands by addition of acetic acid. It is an acidic protein and probably exists in saliva as the potassium salt. On hydrolysis it yields mucoitin sulphuric acid.

The origin of the thiocyanate in saliva is supposed to be from traces of cyanides formed in metabolism. It is not always present. The alleged correlation between this substance and tobacco smoking cannot be substantiated.

The urea and uric acid, and possibly some salts, are probably derived from the blood by diffusion and have no special function. Chloride is important as an activator for ptyalin.

On standing, saliva loses CO_2 and deposits calcium phosphate and carbonate. This is the probable origin of the " *tartar* " of the teeth.

Gastric Juice

Gastric juice is the most acid secretion of the body. The acidity is due to HCl. The strength of HCl secreted by the glands is about 0·55%, which is equivalent to 0·15 N and pH 0·9. The total chloride is 0·165 N. This high acidity of the freshly secreted juice is quickly reduced in the stomach, so that the stomach contents as ordinarily examined show an acidity of from 0·15% to 0·25% HCl. Neutralisation of the acid can be achieved in several ways. The mucin of saliva, being a potassium salt of a weak acid, will take up HCl to form KCl and mucin hydrochloride. The high water content of saliva diminishes the HCl concentration by dilution. The mucus of the stomach also helps to neutralise the acid and may be the major factor in the resting stomach when the secretion of juice is slow. Food proteins combine with hydrochloric acid. There may also be regurgitation of alkaline fluid from the duodenum.

In addition to the HCl, gastric juice contains about 0·55% solids of which a little over 0·4% is organic. This organic portion includes the enzymes pepsin, gastric lipase and rennin (?) and the protein mucin; the inorganic portion consists mainly of the chlorides of Na and K, the latter slightly in excess, with traces of Ca, Mg, phosphate and sulphate.

The amount of juice secreted varies considerably. On an ordinary diet it is supposed that between 2 and 3 litres are secreted daily by an adult.

The Origin of Gastric HCl. Normally the pure gastric juice flowing from the glands has a constant acidity corresponding to 0·15 N HCl. The production of this acid from the parietal cells, which are probably alkaline in reaction, has not yet been satisfactorily explained, although many mechanisms have been proposed.

(1) According to Matthews, HCl might be liberated by hydrolysis of the chloride of a weak base such as ammonia :—

$$NH_4Cl + H_2O \rightarrow NH_4OH + HCl.$$

The ammonia is absorbed in some way, leaving the HCl to be secreted. This is supported by the relatively high concentration of ammonia in gastric mucosa.

(2) A more favoured hypothesis assumes the interaction of sodium chloride with acid phosphate thus :—

$$NaCl + NaH_2PO_4 \rightarrow HCl + Na_2HPO_4$$

followed by $Na_2HPO_4 + H_2CO_3 \rightarrow NaH_2PO_4 + NaHCO_3$.

The acid phosphate may thus be used again and the bicarbonate removed by the blood stream.

(3) Alternatively, NaCl might react with carbonic acid thus :—

$$NaCl + H_2CO_3 \rightarrow HCl + NaHCO_3.$$

This reaction has been carried out experimentally in the presence of protein.

(4) The formation of HCl can be explained physically on the basis of Donnan's theory of membrane equilibrium if it is assumed that chloride is combined in the cell with protein, R (see p. 42).

Original State		*Equilibrium*	
$R^{\cdot}$	$H^{\cdot}$	$R^{\cdot}$	$H^{\cdot}$
Cl'	OH'	Cl'	Cl'
		OH'	

Though all these mechanisms are possible, none can be regarded as proven.

Gastric Analysis. The contents of the stomach are so readily accessible that gastric analyses are commonly employed clinically. The information which can be adduced in this way includes the amount of *free HCl*, *total acidity*, *organic acids* and *enzymes* present ; at the same time, evidence of regurgitation of bile, the presence of blood, excess of mucus and delayed emptying of the stomach may be obtained. Contents of the stomach are withdrawn at intervals by means of a tube which the subject has swallowed previous to the test. (A stomach tube for this purpose consists of a long thin rubber tube with a suitable perforated end which is swallowed so that it lies in the lower part of the stomach ; the other end is connected to a syringe for the withdrawal of samples ; once swallowed, the tube may be retained in the œsophagus and stomach without great discomfort.) It is usual to empty the stomach before the test and then give a test meal of gruel, prepared according to specific instructions. Samples are withdrawn at definite intervals, centrifuged or filtered through muslin, and aliquot portions analysed. The only analysis calling for description here is that indicating the efficiency of the HCl secretion. The HCl secreted by the stomach may be combined with protein of the food (or mucins) and neutralised by bases such as Na or K. That not neutralised in this way remains as free HCl. There is always some neutral (or mineral) chloride in gastric juice and usually some in the food and saliva. The amount of acid other than HCl is normally very small, but appreciable amounts of organic acids such as lactic may be formed from carbohydrate by bacterial action if HCl secretion is deficient (*achlorhydria*). The action of bacteria is normally inhibited by free HCl. We have, then, in gastric contents free HCl, protein hydrochloride, mineral chloride, and possibly organic acid. These can be distinguished by four analyses as follows :—

(1) **Free Acidity.** By using a suitable indicator (Töpfer's reagent or thymol blue) the sample is titrated with N/10 NaOH to about pH 3. At this pH protein hydrochloride and organic acids are barely dissociated, so that only *free HCl* will titrate.

(2) **Total Acidity.** The sample is titrated with N/10 NaOH to pH 9 (phenolphthalein or thymol blue). All the acids are dissociated at this pH, so that this titration gives *free HCl + protein HCl + organic acid.*

(3) **Mineral Chloride.** A sample is evaporated and ignited and the chloride in the residue estimated with N/10 $AgNO_3$ and KCNS. Since HCl (both free and protein HCl) is lost during ignition, this only gives *chloride attached to inorganic base such as Na or K.*

(4) **Total Chloride.** A sample is made alkaline to fix the total HCl and then treated as in (3). This gives *free HCl + protein HCl + mineral chloride.*

From these four results, which are usually expressed as c.c. N/10 acid per 100 c.c., all necessary information can be calculated. Free HCl is given by (1). The difference (4) – (3) gives free HCl + protein HCl, usually called the **active HCl.** Subtraction of the active HCl from (2) gives the organic acids. Normally this should be very small. Normal values for free acidity rise to about 50 c.c. N/10 per 100 c.c (pure gastric juice would be 150 c.c.) and the total acidity about 75 c.c

N/10. These figures show how the gastric juice is diluted and partially neutralised.

Time of Gastric Digestion. The time taken for digestion in the stomach varies according to the nature and quantity of the food. An ordinary meal is evacuated from the stomach in about four hours. The time is lengthened if the food has not been properly masticated, or if there is an excessive amount of fat, or if gastric secretion and activity are suspended by vigorous exercise or emotion.

The actual digestion in the stomach, as will be seen later, is relatively little. The stomach is probably of greater importance in reducing the food to a fairly uniform semi-fluid mass and passing this on to the intestine in regulated amount and at a suitable temperature. The food ejected from the stomach is next subjected to the action of pancreatic juice, bile and intestinal juice.

Pancreatic Juice

Pancreatic juice is a clear alkaline fluid with a pH about 8·0. It contains approximately 1·8% of solids (including HCO_3); 0·6% is organic, comprising proteins, the enzymes trypsinogen, chymotrypsinogen, carboxypolypeptidase, lipase, amylase, maltase and other organic substances. The inorganic material, nearly 1·2%, is mainly Na, Cl and HCO_3 with small amounts of K, Ca and HPO_4, the total base being equivalent to about 160 c.c. N/10 per 100 c.c. Approximately half of this is combined as chloride and the remainder as bicarbonate. Thus there is about 0·7 g. (= 80 c.c. N/10) $NaHCO_3$ in every 100 c.c. pancreatic juice available for neutralisation of HCl of the gastric contents. We saw that the gastric *juice* (= 150 c.c. N/10 acid per 100 c.c.) was diluted and partly neutralised in the stomach by the food and mucin to give an acidity equal to 75 c.c. N/10 acid per 100 c.c., so that pancreatic juice can neutralise at least an equal volume of gastric *contents*.

Intestinal Juice (*Succus entericus*)

Intestinal juice is secreted abundantly from the duodenal mucosa, and in progressively smaller amounts along the jejunum and ileum. In general composition it is similar to pancreatic juice, containing about 1·5% solids, of which nearly two-thirds is inorganic, the remainder consisting mainly of enzymes and protein. Like pancreatic juice, approximately half the inorganic part is $NaHCO_3$ and half NaCl. The pH is about 7·7. The chief difference between pancreatic and intestinal juice is in the nature of the enzymes. Intestinal juice has a large and varied array of enzymes* which can hydrolyse almost any complex constituent of ordinary food with the exception of cellulose. These enzymes include erepsin (polypeptidase + peptidase), lactase, maltase, sucrase, lipase, phosphatase, nucleinase, nucleotidase, nucleosidase, amylase and protease. Another substance of importance is enterokinase, which activates the trypsinogen of pancreatic juice.

In addition to intestinal juice, there is another secretion of intestinal mucosa. This is a continuous small secretion with negligible enzyme content ; it contains chiefly mucin.

Bile

Bile is continually formed by the liver cells and passed to the gall bladder, where its composition is modified (*a*) by addition of mucin and possibly other substances, and (*b*) by removal of water, bicarbonate and chloride by reabsorption by the bladder mucosa. Whereas liver bile contains about 98% water, bladder bile contains 89%. The former is decidedly alkaline, the latter nearly neutral, and sometimes acid. The table (after Harrison. Ref. 3) gives typical percentage values of human bladder and liver bile.

APPROXIMATE PERCENTAGE COMPOSITION OF HUMAN BILE

Constituent	Bladder Bile	Liver Bile
Water	89·0	98·0
Solids	11·0	2·0
Inorganic substances	0·8	0·75
Bile salts	6·0	0·72
Mucin and pigments	3·0	0·4
Cholesterol	0·38	0·06
Fat, fatty acids, etc.	0·82	0·07

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* According to Florey, the only enzymes constantly found in intestinal juice are amylase and enterokinase. The other enzymes are derived from broken-down cells of the mucosa.

Gall bladder bile may be golden-yellow, brownish-yellow or olive-green in colour, dependent on the relative proportions of the bile pigments. It is a viscid fluid with a bitter taste and characteristic smell. The inorganic material is mainly $Na^{\cdot}$, $K^{\cdot}$ and $Ca^{\cdot\cdot}$ as basic, and Cl' and HCO_3' as acidic radicles. The total base is equivalent to about 170 c.c. N/10 NaOH per 100 c.c. of liver bile and may increase to 300 c.c. N/10 in bladder bile. Bile acids contribute to the total acid, especially in bladder bile.

The Bile Pigments, biliverdin and bilirubin, since they are derived from breakdown of hæmoglobin, are described in detail under blood (p. 177).

Bile Salts. The most interesting constituents of bile are the "bile salts," for these are the substances which are mainly responsible for the unique functions of bile in digestion and absorption. Two acids were originally described, **glycocholic acid** and **taurocholic acid,** but these have subsequently been shown to consist of several closely related acids. The original names are still used to indicate the bile acids collectively. In human bile glycocholic acid predominates, there being about three times as much as taurocholic acid. The latter predominates in the bile of some carnivora. The acids are present in bile as dextrorotatory sodium salts. On hydrolysis they yield a **cholic acid** and either glycine or taurine. The two components are connected by a peptide link. The hydrolysis of bile acids can therefore be represented :—

$$C_{23}H_{39}O_3 \,.\, CO \,.\, NH \,.\, CH_2 \,.\, COOH + H_2O \rightarrow C_{23}H_{39}O_3 \,.\, COOH + NH_2 \,.\, CH_2 \,.\, COOH.$$

Glycocholic Acid Cholic Acid Glycine

$$C_{23}H_{39}O_3 \,.\, CO \,.\, NH \,.\, CH_2 \,.\, CH_2 \,.\, SO_3H + H_2O \rightarrow C_{23}H_{39}O_3 \,.\, COOH + NH_2 \,.\, CH_2 \,.\, CH_2 \,.\, SO_3H$$

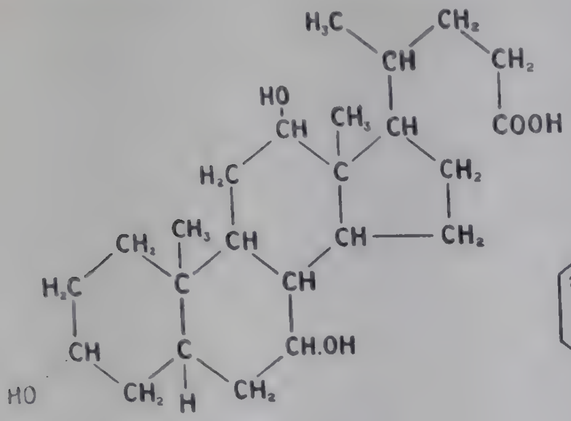
Taurocholic Acid Cholic Acid Taurine

The cholic acids found in human bile are hydroxy derivatives of the saturated **cholanic** acid.

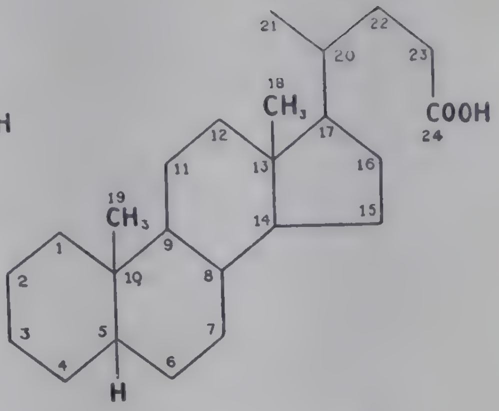
Cholic Acid	3, 7, 12-Trihydroxycholanic acid.
Desoxycholic Acid	3, 7-Dihydroxycholanic acid.
Chenodesoxycholic Acid	3, 12-Dihydroxycholanic acid.
Lithocholic Acid	3-Monohydroxycholanic acid.

Other tri- and dihydroxycholanic acids are found in the bile of different animals.

The formula of cholic acid is :—



CHOLIC ACID



CHOLANIC ACID

The formula of cholanic acid is indicated in the numbered skeleton to the right. For the relationship to cholesterol see p. 87. The origin of the cholic acids in the body is not yet clear ; there is no satisfactory evidence that they are derived from cholesterol. They are probably formed in the liver cells ; when the liver is injured the secretion of bile salts is diminished.

The bile acids have the power of greatly reducing surface tension, thereby assisting the emulsification of fats, but their greatest importance lies in their ability to form hydrotropic compounds (p. 47) with a number of substances, a property which is particularly marked in desoxycholic acid. Many substances, such as fatty acids, phenols, higher alcohols, camphor, naphthalene, combine with desoxycholic acid in various molecular proportions. The resulting compounds are called **choleic acids,** *e.g.*, stearic acid-choleic acid, and are water-soluble even at pH 6. By such combinations insoluble fatty acids, cholesterol, fat-soluble vitamins, drugs (*e.g.*, alkaloids) are rendered soluble and diffusible, and thus capable of being absorbed. The cholesterol in bile is kept in solution by this means. Occasionally concretions (*gall stones*) are formed in the gall bladder ; most gall stones contain cholesterol, which may even be present to the extent of 97%. In such cases the ratio bile acids : cholesterol in the bile is usually less than 8 : 1. Normally this ratio is exceeded.

Functions of Bile. Bile has many functions in digestion and absorption :—

(1) Choleic acid formation by bile acids permits the absorption of many valuable insoluble compounds.

(2) This solvent action assists digestion by facilitating the action of lipase.

(3) The lowering of surface tension by bile salts assists emulsification of fat.

(4) Bile salts are activators of lipase.

(5) Bile salts are reabsorbed from the intestine and pass back to the liver where they stimulate further secretion of the bile (*cholagogue action*).

(6) Bile is an important source of alkali for neutralisation of hydrochloric acid entering the intestine from the stomach. A large amount of the base in bile is combined with weak acid (as bicarbonate and bile salt), so that, although neutral or even acid, bile can effectively neutralise (buffer) strong acids like hydrochloric acid.

(7) Bile is a channel for the excretion of several substances, among them bile pigments, certain drugs, toxins, copper, iron, calcium and some inorganic salts. The cholesterol and other lipides of bile are probably chiefly excretory products.

(8) Bile stimulates peristalsis. (This is disputed.)

(9) The absence of bile seriously impairs digestion, particularly of fat, as might be expected from (1), (2), (3), (4). If fat is not digested it tends to form a layer round particles of other foodstuffs and diminish the surface exposed to enzymes. In this way bile may be said to be important for digestion of all foods, and its absence will upset digestion generally. It has been claimed that in the absence of bile there is more putrefaction in the large intestine.

It has been computed that a man secretes from 500–1,200 c.c. of bile per day.

THE REACTION OF THE INTESTINE

We have seen that hydrochloric acid produced by the stomach can be neutralised by

(1) Mucin of saliva and gastric mucosa.
(2) Proteins and other substances of food.
(3) Bases of pancreatic and intestinal juices and bile.
(4) Proteins in these juices.

The effect of these substances cannot be expressed quantitatively owing to the fundamental difficulty of measuring the amounts of the various secretions under strictly physiological conditions. Values have been calculated, but are based on aphysiological foundations. The ultimate result, however, can be seen by measuring the pH of intestinal contents. Modern determinations leave no doubt that the contents of the small intestine are essentially **acid,** and not, as was supposed (largely to accommodate the idea that fat was absorbed as soaps), alkaline. It is interesting to note that Claude Bernard taught that the reaction of the intestine was generally acid. In man the reaction is nearly always acid and values from pH 4·5 to 7·9 have been observed. Similar values have been obtained in other mammals, rodents and birds. The reaction is rarely more alkaline than pH 7. These acidities are due to weak acids and *not* hydrochloric acid, so that the action of the intestinal secretions is to neutralise hydrochloric acid, but not to make the intestinal contents alkaline. An alkaline reaction in the intestine can only be assumed in the immediate neighbourhood of an alkaline secretion. It should also be pointed out that a certain amount of acid is liberated from certain foods during digestion ; this includes fatty acid from fat, phosphoric acid from phospholipides and phosphoproteins, sulphuric acid from mucoproteins. A consequence of the acidity of the gut is that soaps cannot exist, since soaps of the common fatty acids only show their characteristic properties above pH 8.

Normally the chyme stays in the small intestine from four to six hours. If the food contains substances (*e.g.*, excess of fruits) which increase peristalsis, the time may be diminished with correspondingly incomplete digestion and absorption.

BACTERIAL DECOMPOSITION IN THE INTESTINE (41)

The intestinal contents passing the ileocæcal valve under normal conditions consist of undigested food residues, unabsorbed products of digestion, remains of alimentary secretions and cellular *débris.* The mass contains a large proportion of water and is isotonic with blood, the substances responsible for the osmotic pressure being largely diffusible blood constituents, mainly NaCl. During about thirty-six hours this mass is converted into fæces (p. 459) and four-fifths of the water and the diffusible salts are absorbed ; the pH changes from acid to slightly alkaline (7·0–7·5). During this time several changes take place in the mass, largely owing to the bacteria which flourish in the large intestine.

In the small intestine bacteria are present which attack carbohydrate and produce acids such as lactic, acetic, propionic, butyric. So long as there is sufficient carbohydrate, other bacteria do not appear to be very active. If there is an insufficiency of carbohydrate, bacteria attacking protein become active. In any case, the number of bacteria is small and the time available for action is short, so that there will be relatively little bacterial decomposition. The main seat of bacterial action is the large intestine, where the undigested food residues and alimentary secretions are attacked. The action of bacteria under these conditions is often referred to as *putrefaction*, especially with proteins, and as *fermentation* with carbohydrates.

Bacterial Decomposition of Carbohydrate. Carbohydrates yield a variety of substances, among which are the acids mentioned above. Other products are the gases carbon dioxide, methane and hydrogen. The many products may be produced in so many ways by different bacteria and the reactions involved are so varied that it must suffice to give two reactions, which have been shown to proceed almost quantitatively :—

$$C_6H_{10}O_5 + H_2O \rightarrow 3CO_2 + 3CH_4$$

In this case the carbohydrate is cellulose ; other carbohydrates give hydrogen and but little methane. *Bacillus coli communis*, according to Harden, breaks down sugar thus :—

$$2C_6H_{12}O_6 + H_2O \rightarrow$$
$$2CH_3 . CHOH . COOH + CH_3 . COOH + C_2H_5OH + 2CO_2 + H_2.$$

An organism isolated from human intestine, *Bacillus cellulosæ dissolvens*, attacks cellulose, but not other carbohydrates, giving butyric acid, ethyl alcohol, CO_2 and H_2.

Bacterial Decomposition of Fat. Little is known of the action of bacteria on fat except that glycerol and fatty acids are formed. Bases liberated from phosphatides, *e.g.*, choline, may undergo certain changes involving the formation of more toxic bases, *e.g.*, neurine.

Bacterial Decomposition of Protein. The most characteristic action of bacteria is upon the amino-acids which they liberate from proteins ; this involves removal of amino and carboxyl groups, and may be accompanied by oxidation or reduction. The products formed depend upon the nature of the intestinal contents and the particular bacteria at work, so that the detailed mechanism of normal bacterial degradation of amino-acids in the large intestine is uncertain; if, indeed, there is a normal path.

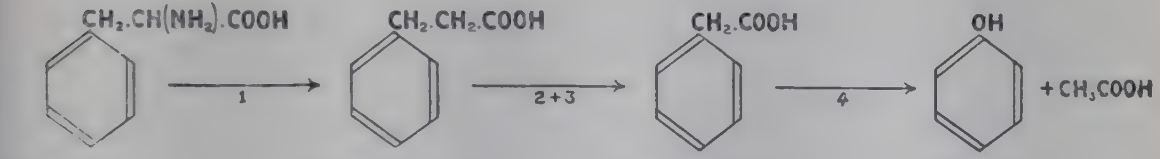
The chief reactions are **reductive deamination** (in contrast with the oxidative deamination in ordinary metabolism, p. 270), yielding a fatty acid,

$$R . CH(NH_2) . COOH \xrightarrow{+ H_2} R . CH_2 . COOH + NH_3$$

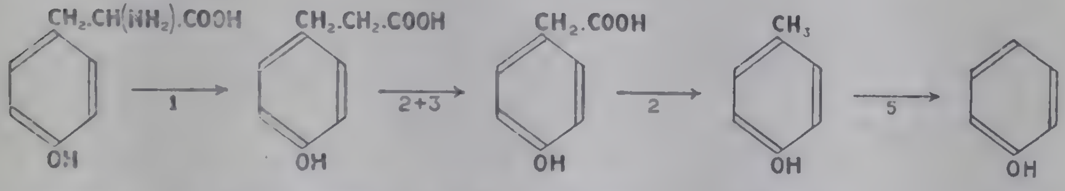
and **decarboxylation**, yielding a toxic amine,

$$R . CH(NH_2) . COOH \longrightarrow R . CH_2 . NH_2 + CO_2.$$

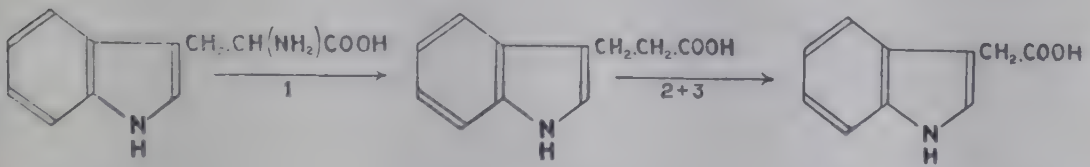
The most interesting products are derived from basic amino-acids and those with aromatic nuclei. Phenylalanine, tyrosine and tryptophan, as a result of both types of reaction, yield phenols. Possible mechanisms, of which the intermediate products are said to have been detected, are set out below.


CH₂.CH(NH₂).COOH
1
CH₂.CH₂.COOH
2+3
CH₂.COOH
4
OH
+ CH₃COOH

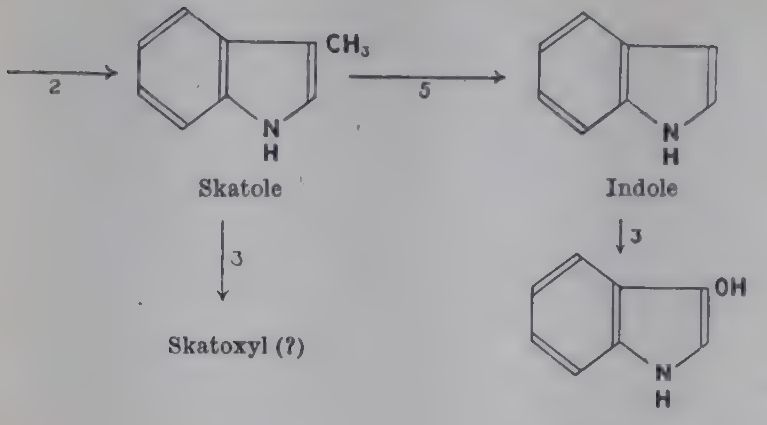
Phenylalanine Phenylpropionic Acid Phenylacetic Acid Phenol


CH₂.CH(NH₂).COOH
OH
1
CH₂.CH₂.COOH
OH
2+3
CH₂.COOH
OH
2
CH₃
OH
5
OH

Tyrosine *p*-Hydroxyphenyl-propionic Acid *p*-Hydroxyphenyl-acetic Acid *p*-Cresol Phenol


CH₂.CH(NH₂)COOH
NH
1
CH₂.CH₂.COOH
NH
2+3
CH₂.COOH
NH

Tryptophan Indole-propionic Acid Indole-acetic Acid



REACTIONS

1. Deamination.
2. Decarboxylation.
3. Oxidation.
4. Hydrolysis.
5. Demethylation.

The evidence for these schemes is not, however, conclusive. Some biochemists dispute the transformation of skatole into indole, and maintain that the change of tryptophan into indole, catalysed by the enzyme tryptophanase, occurs without the formation of detectable intermediate products.

The phenols, normally formed in small amounts and only partly absorbed, are readily detoxicated in the body (p. 316). Indoxyl and skatoxyl * are not actually formed in the large intestine, but in the body from absorbed indole and skatole as a preliminary step in detoxication.

Indole and skatole have a most intense fæcal odour, and it is to these substances that the characteristic smell of fæces is due. About 50 mg. of indole are excreted daily in fæces.

More harmful are the toxic amines formed by direct decarboxylation, although they are probably mostly destroyed before absorption (p. 210). These bases are the so-called *ptomaine* poisons formed when food, especially meat, putrefies. The most poisonous, and the amino-acids from which they are formed, are set out below.

$$NH_2.(CH_2)_3.CH(NH_2).COOH \longrightarrow NH_2.(CH_2)_3.CH_2.NH_2$$

ORNITHINE (from Arginine) PUTRESCINE

$$NH_2.(CH_2)_4.CH(NH_2).COOH \longrightarrow NH_2.(CH_2)_4.CH_2.NH_2$$

LYSINE CADAVERINE

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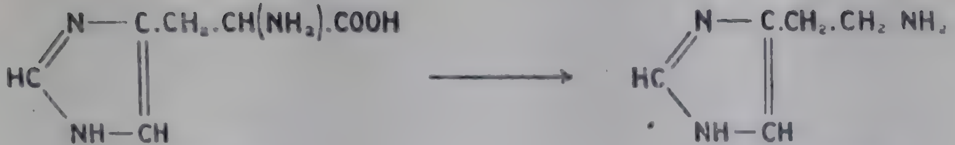
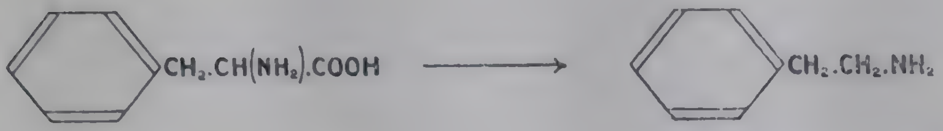
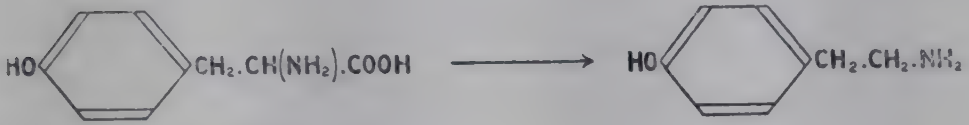
* Skatoxyl is said to be excreted after administration of skatole. Its constitution (if it exists) is unknown.



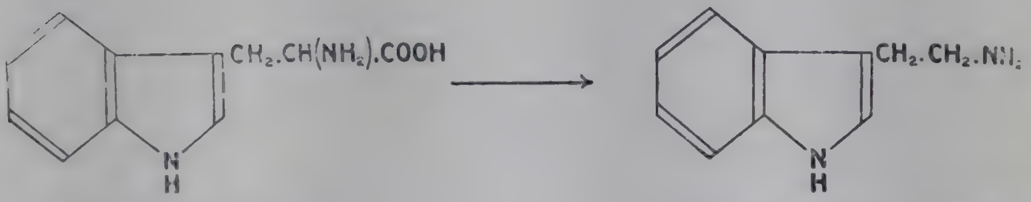
HISTIDINE HISTAMINE



PHENYLALANINE PHENYLETHYLAMINE



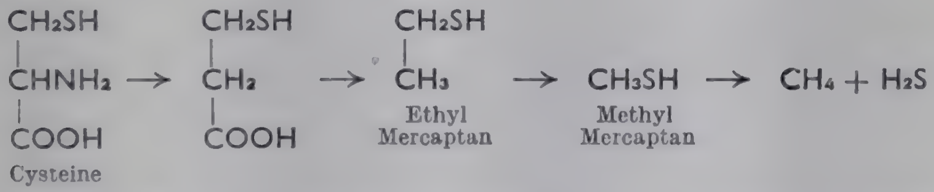
TYROSINE TYRAMINE



TRYPTOPHAN INDOLE-ETHYLAMINE

Tyramine and histamine are powerful pressor and depressor substances respectively.

Cysteine behaves abnormally in giving H_2S ; a possible mechanism is :—



$$\begin{array}{c}CH_2SH\\|\\CHNH_2\\|\\COOH\\\text{Cysteine}\end{array} \rightarrow \begin{array}{c}CH_2SH\\|\\CH_2\\|\\COOH\\\ \end{array} \rightarrow \begin{array}{c}CH_2SH\\|\\CH_3\\\text{Ethyl}\\\text{Mercaptan}\\\ \end{array} \rightarrow \begin{array}{c}CH_3SH\\\text{Methyl}\\\text{Mercaptan}\end{array} \rightarrow CH_4 + H_2S$$

Reduction in the Large Intestine. The lower bowel is the site of several reductions. Biliverdin and bilirubin are reduced to urobilinogen and stercobilin, cholesterol to coprosterol, cystine to cysteine, sulphur to H_2S, bismuth salts to the black BiO and ferric salts to ferrous.

CHAPTER XV

THE BIOCHEMISTRY OF ABSORPTION (GENERAL PRINCIPLES *) (39, 40, 42)

THE extent of absorption of foodstuffs in the stomach is normally so small that it can for practical purposes be neglected. Even if completely digested foods are ingested, *e.g.*, glucose or amino-acids, there is very little absorption. The only article of diet readily absorbed by the stomach is alcohol. This is the reason for the rapid physiological response to alcohol ; if it were absorbed like other substances, it would be necessary to take before dinner cocktails at teatime. Certain drugs and poisons are absorbed by the stomach and even in the mouth, *e.g.*, nicotine.

Food is almost entirely absorbed from the small intestine, an organ anatomically well developed for the purpose. The small intestine in man is about 27 ft. long and has an absorbing area, when its folds and the villi are considered, which has been calculated as about 10 sq. metres. The peristaltic movements of the intestine provide for the thorough mixing of the contents, so that every part will be brought in turn into contact with the absorbing surface. The food remains in the small intestine from four to six hours.

When the histological structure is recalled, it is to be expected that absorption will not proceed at a uniform rate throughout the small intestine. But, apart from the histological differences, there are several factors which must affect the rate of absorption, among them the extent of digestion, the time the digested products are in contact with the intestine and the actual mechanisms of absorption of the substances concerned. It is now certain that absorption is not in every case merely a diffusion of crystalloids through a membrane. Some substances, *e.g.*, glucose and fat, largely depend upon specific activity of the mucosal cells for their absorption, so that we have, in effect, specific absorption of certain substances. Other substances apparently pass the cell walls by simple diffusion. Selective absorption of certain ions can be postulated on the basis of Donnan's theory of membrane equilibrium. It is, therefore, to be expected not only that different substances are absorbed at different rates (irrespective of their molecular size), but also that the same substances are absorbed at different rates in different regions of the small intestine. Amino-acids and sugar are said to be absorbed more quickly (per unit area) in the duodenum and progressively less down to the ileum ; bile salts, on the other hand, are absorbed most rapidly in the ileum and least in the duodenum.

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* Details of the absorption of particular substances are described under the utilisation of those substances.

The process of absorption is not regulated to the needs of the animal. The various absorptive mechanisms can apparently work to capacity regardless of whether the substance being absorbed is needed or is in excess of requirement. Useless (*e.g.*, xylose) or even toxic substances are absorbed if they are diffusible.

One component of the chyme may influence the absorption of others. Amino-acids inhibit each other's absorption and glycine reduces the absorption of glucose. Galactose is absorbed more quickly than glucose when the sugars are given separately, but if mixed, glucose is absorbed more rapidly. Further, substances such as water, salts and sugar may pass through the mucosa of the small intestine from the blood into the intestine when the relative concentrations of the blood and intestinal contents are suitable.

Owing to the many factors concerned, the experimental investigation of absorption is beset with difficulty. Many early experiments must be discounted on the grounds of being unphysiological. Modern researches have thrown considerable light on the mechanisms of the absorption of particular substances (see Chapters, XVII, XVIII, XIX, XXIII). The chief methods employed have been :—

(1) Feeding strong solutions of the substance to starved rats by stomach tube and subsequently killing the rat and excising the gastro-intestinal tract for analysis of unabsorbed material.

(2) A loop of intestine of an anæsthetised animal is washed out with saline and test substances injected with a syringe. After a suitable interval the animal is killed and its intestine washed out for analysis.

(3) London's angiostomy method consists in arranging so that

periodic samples can be taken directly from selected points in the circulation of an intact animal.

The Mechanisms of Absorption

Many substances are absorbed by simple diffusion in contrast to the special mechanisms of selective absorption. When substances are absorbed by simple diffusion, the rate depends upon the molecular size of the substances. If a substance is absorbed at a *greater rate* than other substances of similar molecular size, we must assume a *special mechanism of selective absorption.* Galactose and glucose are absorbed at about three times the rate of pentoses ; this is due to acceleration of the diffusion process by chemical reactions (p. 218). Fatty acids, being insoluble, would hardly be expected to diffuse at all ; these are rendered diffusible by forming hydrotropic compounds with bile salts (p. 244). Calcium salts are similarly treated.

To return to simple diffusion, we have a system which can fundamentally be represented :—

INTESTINAL CONTENTS	‖	BLOOD PLASMA
Lumen	Mucosa	Capillaries of Villi

The intestinal contents might be hypertonic, isotonic or hypotonic with the blood plasma. At first sight it might be thought that substances could only be absorbed from hypertonic solutions and that, when isotony is attained, absorption will cease. That, however, would imply that the mucosal membrane is only permeable in the direction lumen → capillaries. Actually this is not so.

Isotonic solutions of some sugars injected into intestinal loops rapidly diffuse into the blood, although the solution in the loop remains isotonic and does not at first diminish in volume ; the lost sugar is replaced by an isotonic amount of " NaCl " * from the blood. If the blood is suddenly rendered hypertonic, as by injection of strong sugar solution, some sugar is excreted into the lumen of the intestine.

The absorption of hyper-, iso- and hypotonic solutions is well exemplified by some recent experiments by Verzár and McDougall, in which various substances were injected into intestinal loops of anæsthetised rats and the residue analysed after one hour and at intermediate periods. The substances investigated were NaCl, glucose and the pentose xylose. Of the sugars only the former is selectively absorbed. (It must be remembered that the experiments were not carried to complete absorption.)

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* It is convenient here to use " NaCl " to represent the easily diffusible substances of blood.

NaCl. Hypotonic solutions of NaCl were rapidly absorbed, including the water. From hypertonic solutions, NaCl diffused out until the solution became isotonic ; practically no water was absorbed.

Glucose. Isotonic glucose was all absorbed. During the process about half the water was absorbed. Throughout the experiment the solution in the intestine remained nearly isotonic, " NaCl " diffusing into the lumen. In the absorption of hypertonic glucose, at first glucose was absorbed and at the same time water was added to the intestine until isotony was attained, when absorption proceeded as above. At the end of the hour about 60% glucose and all the increased water had been removed.

Xylose. At the end of one hour only about 50% of xylose was absorbed from isotonic solution, but no water. More " NaCl " diffused into the intestine than was necessary to attain isotony, so that, since the final solution was isotonic, the volume had increased. With hypertonic solutions there was a big increase in volume (66%) and entrance of " NaCl " ; the intestinal contents became isotonic. Only 30% of xylose had been removed.

That the difference in the results with glucose and xylose is due to the selective mechanism of glucose absorption was shown by obliterating this mechanism by poisoning the rats with monoiodoacetic acid (p. 219). Under these conditions the absorption of xylose was unaffected and the absorption of glucose became similar to that of xylose.

These results are compatible with osmotic laws. It must be remembered that the concentration of the solutes must be considered as well as the total osmotic pressure, and that *the blood rapidly disposes of absorbed products.* Water would be rapidly absorbed from hypotonic solutions. When isotony is attained, the concentration of solute is greater than in blood, so that it will diffuse into the blood, making the solution again hypotonic, providing that the solute diffuses *quickly*, and water will be again absorbed, and so on. In this way a hypotonic solution would be rapidly absorbed. Isotonic solutions of rapidly diffusible substances would likewise be rapidly absorbed. This explains the behaviour of hypo- and isotonic NaCl and glucose solutions. If the diffusion rate is appreciably *slower* than that of the "NaCl" of blood, as in the case of xylose, water may be absorbed from a hypotonic solution ; when the solution becomes isotonic, xylose will diffuse into the blood, but *not* water, since "NaCl" diffuses out of the blood faster than xylose goes in, so that the solution remains isotonic and may even get slightly hypertonic, when water will diffuse out of the blood to regain isotony ; and so the absorption of a slow-diffusing substance like xylose will be slow, and, if it be completely absorbed, will leave in the lumen an isotonic solution of "NaCl." Hypertonic solutions will become isotonic by loss of solute and gain of water ; in the case of xylose more water will be gained, since xylose diffuses slowly and some "NaCl" may be gained.

The rate of absorption of ordinary substances will depend upon the rate of diffusion of the molecules compared with "NaCl." In special cases such as glucose, a special mechanism may remove the absorbed substance as soon as it passes the membrane and so greatly enhance its diffusion rate by maintaining a big concentration difference between the two sides of the membrane.

Now by this osmotic explanation of absorption we are left with a residue of isotonic "NaCl," which we know is absorbed somehow. This also can be explained, as Starling pointed out, on osmotic principles, although it is more probably absorbed in the large intestine by filtration.

The diffusible constituents of blood are not the only ones contributing to its osmotic pressure. The plasma proteins exert a small but significant osmotic pressure, so that blood will have a higher osmotic pressure than the intestinal contents, even when the diffusible constituents are in equilibrium, for soluble colloids are only present in such intestinal contents in relatively small amount. Therefore water will pass into the blood ; it follows that the diffusible constituents in the lumen will become more concentrated than those in blood ; therefore salts will diffuse into the blood. In this way complete absorption could be attained. How much is absorbed in this way is not known.

By way of summary let us consider the absorption of ordinary food which, when it enters the small intestine, is largely colloidal. The chyme will be hypotonic. As amino-acids, sugars and other diffusible substances are liberated in digestion they will be absorbed along with water, either by simple diffusion or at an enhanced rate owing to some specific chemical process in the mucosal cells; sparingly soluble substances like fatty acids are rendered diffusible by special processes, such as the formation of hydrotropes. At the same time diffusible constituents of the blood may enter the lumen, so that eventually, when diffusible substances are no longer liberated from colloids, a condition of isotony will be attained. This would normally be the condition of the food residue passing the ileocæcal valve. The bulk of the salts and water are regained by the body from the large intestine.

Surface activity, electrical forces, changes in membrane permeability and pumping action of the villi may play some, but probably a very small, part in absorption. The main force of absorption is undoubtedly osmotic, assisted by the special mechanisms, *e.g.*, for glucose, fatty acids, calcium (Chapters XVII, XVIII, XXIII), which account for the so-called "*vital activity*" or "*power of selective absorption of the mucosa*." (There is no selective absorption from dead intestine.) It must, however, be admitted that there are apparent contradictions, although these can be countered by plausible argument; further, Schreinemakers has shown that the laws of osmosis where several solutes are concerned are extremely complex, so that results apparently contradictory to ordinary osmotic laws are obtained even with dead membranes.

ABSORPTION FROM THE LARGE INTESTINE

In the large intestine filtration is probably the most important method of absorption. In the small intestine there is no appreciable hydrostatic pressure except a transitory one during a peristaltic wave. In the large intestine a pressure generated can be maintained, since it is virtually a tube closed at both ends. There is a positive pressure, which may reach 400 mm. H_2O in the dog, and which is only released on defæcation. It has been shown experimentally that absorption of isotonic solutions is proportional to the intra-intestinal pressure.

The substances absorbed from the large intestine include about four-fifths of the water of the chyme and soluble salts, which have diffused into the chyme from the blood, and various diffusible products of bacterial action. The bacterial products include phenol, cresol, indole and skatole and the intermediate products of their formation such as phenyl-, *p*-hydroxyphenyl- and indole-acetic and propionic acids, histamine, tyramine, putrescine and cadaverine derived from protein and various simple fatty acids and related compounds derived from carbohydrate. It should, however, be remembered that, in spite of the time the food residues are in the large intestine, only very small amounts of these substances are absorbed, if any,* partly because they are not present in the lumen in large amounts and partly because the large intestine is not a very efficient absorbing organ. It can only absorb about 6 g. of glucose per hour. (The value of rectal feeding depends upon the nutrient enemata reaching the *small* intestine through the ileocæcal valve. Only water, alcohol and certain salts are easily absorbed from the large intestine.) In man the large intestine is probably more important as an excretory organ, since it plays an important part in the excretion of Ca, Mg and heavy metals, *e.g.*, bismuth and mercury.

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* Histamine, for example, is destroyed in the intestine ; tyramine is probably converted to *p*-hydroxyphenylacetic acid.

CHAPTER XVI

THE USE OF ISOTOPES IN BIOCHEMICAL INVESTIGATIONS (92, 93, 96)

The use of isotopes for the investigation of problems of metabolism has received considerable attention in recent years. Small concentrations of isotopes are metabolised by living cells in the same way as the corresponding ordinary elements. Thus the substitution of certain atoms in a compound by isotopes provides a convenient way of marking groups or compounds for experimental study. Previous to the use of isotopes the only method of labelling a compound was by substituting another element (*e.g.*, halogen for hydrogen in fat studies, p. 247); this was open to objection since the labelled compounds were chemically and physiologically different from the compounds which they were intended to imitate. Provided that the isotope marked compounds used contain only small amounts of the isotope they can be regarded as having the same physiological properties as the normal compounds. Fortunately sensitive methods are available for the estimation of small quantities of isotopes.

The general principle of isotopic investigation is that the substance to be investigated is synthesised with one or more of its atoms having an abnormal isotopic composition. If, after administering such a compound, another compound with abnormal isotopic composition is isolated from the tissues or from urine, then it can be inferred that a chemical conversion of one into the other has occurred.

There are, however, many difficulties in isotopic experimentation. The isotopes themselves are usually only prepared with considerable difficulty. The forms in which they can be prepared are limited. This often imposes extra labour in the synthesis of the compounds required for an investigation, *e.g.*, nitrogen labelled compounds must be synthesised from isotopic ammonia and carbon labelled compounds from isotopic carbon dioxide. Special skill and apparatus beyond the scope of the ordinary laboratory are required for the estimation of the isotopes. Great care is necessary in the selection of the isotopes and the compounds to be used, for not all compounds marked with isotopes are stable. The isotope of hydrogen, deuterium, is stable, for example, when linked to carbon as in $-CH_3$ or $=CH_2$, but when in polar groups such as $-OH$, $-COOH$, $-NH_2$ it readily interchanges with the hydrogen of water. Deuterium obviously cannot be employed for marking the latter groupings since the marker will be lost to water shortly after ingestion. Deuterium attached to carbon adjacent to a carbonyl group is also unstable owing to enolisation

$$-CH_2.CO- \longrightarrow -CH{:}COH- .$$

In spite of the great difficulties involved much valuable work has been done with isotopes especially in the study of the metabolisms of lipides and proteins. These experiments have, in general, confirmed existing knowledge of metabolic processes; they have, above all, emphasised that large molecules and their component units, fatty acids, amino-acids, etc., are constantly involved in rapid chemical reactions. It is clear that peptide, ester and other linkages are continuously being broken and reformed and that the compounds in the body are in a rapid state of flux. To quote Schoenheimer (Ref. 96, p. 63), the chief pioneer in this field: "Components of an animal are rapidly degraded into specific molecular groupings, which may wander from one place to another. The chemical reactions must be balanced so delicately that, through regeneration, the body components remain constant in total amount and in structure. This constancy is not to be taken as an indication that the structural matter of the living organism is inactive and takes little part in metabolism."

Hevesy, in 1923, was the first to realise the practical value of isotopes for the investigation of biochemical problems. He employed the radioactive isotope of lead to study the fate of that element in the body. Urey's discovery of deuterium in 1932 was followed by its production on a practical scale along with the isotopes of the other elements of organic compounds, carbon, nitrogen, oxygen and sulphur. This opened the way for Schoenheimer and others to a very wide application of the isotopic method to metabolic problems.

Stable isotopes are prepared by fractionation of the naturally occurring mixture. The relative amounts of the different isotopes are given in the table.

Isotope	Abundance (per cent.)	Isotope	Abundance (per cent.)
H^1	99·98	O^{16}	99·81
H^2	0·02	O^{17}	0·03
C^{12}	98·9	O^{18}	0·16
C^{13}	1·1	S^{32}	96
N^{14}	99·62	S^{33}	1
N^{15}	0·38	S^{34}	3

Of these isotopes only that of hydrogen has received a name, *deuterium*, and a symbol, D. The isotopes are sometimes referred to as *heavy hydrogen*, *heavy nitrogen*, and deuterium oxide, D_2O, is known as *heavy water*. Heavy water is present in all samples of water obtained from natural sources and is most conveniently prepared by electrolysis. H_2O is preferentially decomposed so that the residue after electrolysis of a large volume of water is a relatively concentrated solution of D_2O, which can be separated by careful fractionation. That there are distinct differences in properties of H_2O and D_2O is shown in the table.

Property	*H_2O*	*D_2O*
Molecular weight	18	20
Specific gravity, 25° C.	1·0	1·1056
Freezing-point	0° C.	3·8° C.
Boiling-point	100° C.	101·4° C.
Temperature of maximum density	4° C.	11·6° C.
Surface tension (dynes per cm. at 20° C.)	72·7	67·8
Solubility of NaCl in	35·9%	30·5%

(Data from Ref. 15.)

The amount of D_2O in ordinary water varies according to the source. Most samples of tap water contain about 1 part D_2O in 9,000 H_2O. An environment of concentrated or pure D_2O is lethal to small aquatic forms such as protozoa, tadpoles or goldfish ; low concentrations are apparently harmless. The water of the body contains some D_2O, but it is not known whether it has any special function, nor is it known whether it is essential to life. At the present time the chief biochemical interest in deuterium lies in its possibilities as a weapon of investigation.

Isotopes of nitrogen and oxygen are obtained like D by fractionation of the natural mixture, but not so easily. N^{15}, in particular, has been of great value in studying protein metabolism.

The heavy isotopes can be estimated accurately by physical methods. The deuterium content of a substance is determined by oxidising to water and measuring the density or refractive index. To estimate N^{15} the substance is converted to ammonium sulphate by the usual Kjeldahl digestion, nitrogen is liberated from the ammonium sulphate by sodium hypobromite and its N^{15} content determined by the mass spectrograph.

Another type of isotype is the radioactive form of an element prepared artificially, *e.g.*, radioactive carbon, phosphorus, sulphur sodium, potassium, calcium, iron and iodine. Radioactive P^{32} is obtained by neutron bombardment of sulphur. Only those radioactive elements which have a sufficiently long half-life are suitable for metabolic studies. Radioactive isotopes may be estimated by the Geiger counter.

Examples of the use of isotopes for elucidating details of metabolism are more appropriately described when the particular

Isostope	Used in Study of	Page
D	Cholesterol metabolism	257
D	Conversion of phenylalanine to tyrosine	278
D	Desaturation of fatty acids	251
D	Formation of creatine	286
D	Interconversion of amino-acids	393
D	Transmethylation	278
D	Utilisation of depôt fat	247
N^{15}	Essential amino-acids	392
N^{15}	Exogenous and endogenous metabolism	265
N^{15}	Formation of amino-acids from ammonia	297
N^{15}	Formation of ammonia from amino-acids	274
N^{15}	Formation of creatine	286
N^{15}	Transamination	273
N^{15}	Urea formation	269
Radioactive C^{11}	Urea formation	269
C^{13}	Urea formation	269
Radioactive P^{32}	Phosphorus in bone	306
Radioactive P^{32}	Phosphorylation of fat during absorption	244
Radioactive P^{32}	Phosphorylation of fat in the liver	250
Radioactive S^{35}	Formation of cystine from methionine	278

phases are being discussed. For the reader's convenience references to the examples given in this book are recorded in the table shown on p. 214.

There are many other examples of the biochemical application of isotopes, *e.g.*, see Refs. 92, 93, 96.

CHAPTER XVII

UTILISATION OF CARBOHYDRATES

(1, 3, 4, 8, 34, 36, 39, 43, 44, 100)

Carbohydrate forms the main bulk of the food in this country. It has the advantage of being cheap, easily digested and rapidly metabolised. Its presence is essential for the complete oxidation of fat. Carbohydrate metabolism is essentially the metabolism of glucose. Cellulose is hardly attacked in man and will not be considered here.

DIGESTION

Saliva contains the enzyme **ptyalin,** which is secreted at approximately its optimum pH 6·9. In the mouth, however, there is scarcely time for the food to be more than intimately mixed with ptyalin. The digestion of starch (for starch is practically the sole polysaccharide of the food) by ptyalin takes place in the stomach until such time as it is arrested by the food mass becoming permeated with the HCl of the gastric juice. The movements of the stomach tend to keep food which has just entered in the centre of the mass. It is usually estimated that ptyalin digestion may last about half an hour. In this time starch is hydrolysed chiefly to achroodextrin ; some maltose may be formed, since this sugar appears very early in the breakdown of starch. The end products will, of course, depend upon the time the digestion proceeds and the amount of enzyme, *i.e.*, whether the food has been well mixed with ptyalin by thorough mastication or swallowed with little admixture with saliva. The maximum possible digestion of starch by saliva is to maltose.* Ptyalin will not hydrolyse maltose to glucose. The rapid appearance of maltose in the early stages of digestion suggests that it is split off from the large molecules, so that the breakdown of starch by ptyalin can be represented :—

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* The hydrolysis of starch by amylases is a very complex process. *In vitro* often only 80% of the starch is hydrolysed to maltose, the residue being referred to as "limit" or "stable" dextrin. This dextrin is hydrolysed if certain activators are added (see Ref. 20).

STARCH → SOLUBLE STARCH → ERYTHRODEXTRIN
(+ Maltose)
→ ACHROODEXTRIN → MALTOSE
(+ Maltose)

On passing to the duodenum the partially digested carbohydrate is acted upon by the amylase, **amylopsin,** of the pancreatic juice (optimum pH 7). The pancreatic amylase has the same function as the salivary, and starch will be almost completely digested to maltose. Some glucose may be formed by pancreatic maltase, though the major digestion of maltose is by intestinal maltase.

Intestinal juice contains an **amylase** to deal with any undigested polysaccharides and, more important, enzymes for the final digestion of sugars to monosaccharides, **lactase, maltase** and **sucrase** (*invertase*) acting upon lactose, maltose and sucrose (cane sugar) respectively. Lactase is more abundant in young animals than adults. By these enzymes the carbohydrate of the food is broken down into glucose, galactose and fructose. Sugars are also liberated from glycolipides, certain proteins and glycosides by the action of appropriate enzymes, *e.g.*, glucose, galactose, mannose and their aminohexoses, ribose and desoxyribose ; the amounts, however, are small, and we can restrict our study to the absorption of glucose, galactose and fructose. All disaccharides must be broken down, for if they are absorbed they are treated as foreign substances and excreted unchanged ; they cannot be hydrolysed in the body.

ABSORPTION

It is usually taken that sugar is absorbed entirely into the capillaries of the villi and passes to the liver by the portal vein. Actually very small amounts may also be absorbed by the lymph. The passage of sugars through the epithelial wall cannot be entirely a process of simple diffusion for several reasons. If it were we should expect

(1) All hexoses to be absorbed at the same rate.

(2) Pentoses, having smaller molecules, to be absorbed more quickly.

(3) Disaccharides to be easily absorbed.

Actually a large number of experiments on various animals leaves no doubt that different sugars are absorbed at different rates in the *living* intestine; our expectations are only fulfilled in the dead intestine. Galactose and glucose are absorbed at about three times the rate of most other sugars. Values for the proportional absorption rates of pure sugars in rats obtained by Verzár are :—

Galactose	Glucose	Fructose	Mannose	Xylose *
115	100	44	33	30

In mixtures of sugars, galactose and glucose are always more rapidly absorbed than the other components of the mixture.

Another important factor in absorption is the concentration of the sugar. Here, again, galactose and glucose are unique, for the amount absorbed in a given time is the same for almost any concentration; to give a concrete example, 10 g. of glucose would be absorbed in the same time whether it was administered

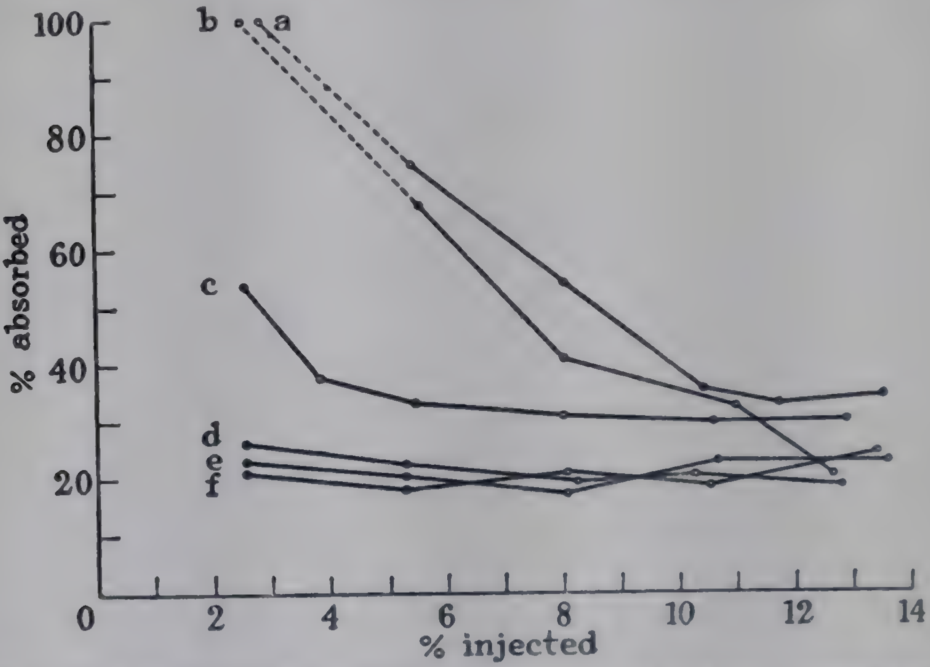

FIG. 18. Relation of percentage absorption per hour to concentration of different sugars injected. (*a*), Glucose; (*b*), Galactose; (*c*), Fructose; (*d*), Xylose; (*e*), Sorbose; (*f*), Mannose.
(From Verzár and McDougall "Absorption from the Small Intestine," p. 125 (Longmans), 1936.)
Note that with glucose and galactose the *amount* absorbed is roughly the same at any concentration.

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* Xylose is a pentose derived from threose.

in the form of 500 c.c. of 2% or 100 c.c. of 10% solution; the absorption of xylose, on the other hand, depends on the concentration of the solution given, so that 100 c.c. of a 10% solution would be absorbed in about one-fifth of the time taken to absorb 500 c.c. of 2% solution. If the absorption were a process of pure diffusion, we should expect greater absorption the greater the concentration of solution administered. This, in fact, occurs with mannose and xylose, and can be seen in the curves (Fig. 18), which were obtained by injecting the sugar solutions into intestinal loops in rats and testing the amount absorbed after a given time. Note that fructose occupies an intermediate position. The selective absorption of galactose and glucose in the living gut strongly suggests that some chemical process is involved. A clue to the nature of this process was provided by studying the effect of certain poisons—in particular, monoiodoacetic acid and phlorrhizin, which were known to affect the reabsorption of glucose in the kidney. Iodoacetic acid injected subcutaneously into rats was shown to diminish the rate of absorption of glucose relative to that of xylose; glucose and xylose mixed, which were normally absorbed in the ratio 4·5 : 1, were then absorbed at equal rates. Now these poisons are known to inhibit phosphorylation, and there is evidence that galactose and glucose are combined with phosphoric acid to form hexosephosphate in the mucosa. The two sugars absorbed in this way are those whose utilisation in the body involves phosphorylation (*e.g.*, muscular contraction). The other sugars which appear to be absorbed by diffusion alone are neither phosphorylated nor appreciably utilised in the body. The intermediate behaviour of fructose is explained by its partial conversion to glucose, a change which can be shown to occur in the isolated intestine or even under the influence of an extract of mucosa (*i.e.*, the mucosa contains an enzyme capable of converting fructose to glucose); the process is, however, relatively slow, since fructose is not absorbed at the same rate as glucose.

The accelerating action of the phosphorylation is not due to increased diffusibility of the hexosephosphate, but probably to the effective removal of free hexose as soon as it diffuses into the epithelial cell. The hexosephosphate is apparently broken down again before entering the blood stream, which takes up the free hexose and not hexosephosphate.

It has been suggested that the phosphorylation process may be controlled by the hormone of the adrenal cortex, for glucose, but not xylose, absorption is diminished in adrenalectomised rats. The normal glucose absorption returns on administration of cortical hormone. Recent work, however, has failed to confirm these experiments.

INTERMEDIARY METABOLISM

The amounts of galactose and fructose absorbed are normally small, and, since these sugars are apparently converted to glucose as soon as they reach the liver, we need only consider the fate of glucose. One difficulty in studying carbohydrate metabolism is that no end product other than CO_2 and H_2O is excreted. Excessive administration of glucose does not result in excretion of a partially oxidised glucose, but in glucose itself. We must, therefore, examine variations in the sugar content of the blood. Many difficulties have arisen here, because all suitable methods of estimating small amounts of sugar depend essentially on measuring reducing power. Now glucose is not the only reducing substance in blood, and it is only recently that methods of determining " true " blood sugar have been developed. Since, however, the other reducing substances, *e.g.*, glutathione, remain fairly constant, the old methods reflect changes in blood sugar with fair accuracy. The normal value for true blood sugar in man is about 80 mg. per 100 c.c. ; this is fairly equally distributed between corpuscles and plasma,* and is mostly α and β-*d*-glucopyranose. It is still customary to record the reducing value as the blood sugar, *i.e.*, 100 mg. per 100 c.c.

Glucose Tolerance. Given by mouth it should be possible for a healthy man to take 150 g. of glucose without *glycosuria* (sugar in urine). More than this amount may cause slight glycosuria. The tolerance for other sugars is different, usually lower. Such figures, however, will give us little useful information, since, when large quantities of sugar are ingested, the emptying of the stomach, and, therefore, absorption is delayed. If glucose be injected intravenously, it is found that up to 0·9 g. per kg. body weight per hour may be administered continuously to man, dog or rabbit without causing glycosuria. Amounts above this cause slight glycosuria. Thus theoretically an 11-st. man could use about 1,500 g. a day without causing glycosuria ; this is an ample margin over the amount of carbohydrate normally ingested (about 500 g.). Actually carbohydrate is eaten mainly in the form of starch, for which there is apparently unlimited tolerance. This must mean therefore that the rate of digestion and absorption of starch does not exceed the intravenous tolerance, *i.e.*, 0·9 g. per kg. body weight per hour.

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* In other species the plasma sugar is generally the greater.

The Fate of Glucose in the Liver

The importance of the liver in carbohydrate metabolism was realised by Claude Bernard in 1850, when he discovered glycogen in the liver and muscles. He observed the rapid *post-mortem* conversion of liver glycogen into glucose, and explained the function of the liver in storing glucose as glycogen. We now know that the hexoses in the portal vein pass into the liver, where they may be stored as glycogen or passed into other tissues for utilisation. Glucose may be released into the circulation from the glycogen as required. The importance of this may be seen in hepatectomised animals; if sufficient glucose is not continuously injected into the blood to maintain a normal blood sugar value, the animal dies in a very short time, in spite of the fact that the muscles contain a good supply of glycogen. This shows that the blood must be supplied with glucose and that the liver is probably the only organ which can do this. Under normal conditions the blood sugar level varies within narrow limits (80–180 mg. per 100 c.c. blood in man) and depends upon the amount of glucose which enters and leaves the blood stream. In the fasting animal the inflow equals the outflow and the blood sugar is steady. Sugar is provided by the liver to replace that used by the tissues, for the blood sugar of the hepatic vein is higher than that of the portal vein or hepatic artery. After a meal the blood sugar in the portal vein rises, but normally the general blood sugar is not greatly raised (120 mg. per 100 c.c.), although considerable amounts of carbohydrate may have been taken. This influx of sugar can be disposed of by (1) storage as glycogen in the liver; (2) storage as glycogen in other tissues, *e.g.*, muscles; (3) oxidation; (4) conversion into fat. If these paths will not accommodate the flood of sugar the excess will be excreted, but this only occurs when the glucose tolerance is exceeded. Glycosuria in the healthy man is an emergency device analogous to a sluice gate, for it only occurs if the blood sugar level has been raised above about 180 mg. per 100 c.c. Since at blood sugar values below this all sugar is reabsorbed in the kidney, this value (180 mg.) is called the **Renal Sugar Threshold** (R.S.T.).

As might be expected, there is a difference between the arterial (or capillary) and venous blood sugar values, indicating absorption of sugar by the tissues. Under fasting conditions the difference is so small (2 or 3 mg. per 100 c.c.) that it can be neglected for practical purposes, *i.e.*, blood for analysis may be taken either from finger capillaries or a vein. During absorption of large amounts of sugar, however, big differences (40–60 mg.) may be observed.

Glycogenesis in the Liver

The process of the formation of glycogen in the body is known as *glycogenesis*. While under normal conditions the glycogenesis is very largely from glucose, a number of substances may be synthesised to glycogen, among them galactose, fructose, mannose, glycerose, dihydroxyacetone, methyl glyoxal, pyruvic acid, lactic acid, glycerol and several amino-acids, *e.g.*, alanine, glutamic acid and glycine. The term *glyconeogenesis* is used to denote the formation of glycogen from non-carbohydrate sources. Of the fatty acids only those with an odd number of carbon atoms like propionic acid form glycogen in the liver. Pentoses do not form glycogen. The non-carbohydrate substances are particularly important in starvation or in carbohydrate deficient diets. Even in prolonged starvation glycogen is always found in the liver, although the level, like the blood sugar level, is below normal ; in these circumstances the glycogen is formed from tissue protein and the glycerol portion of fat through the intermediates mentioned above. The synthesis of glycogen from glucose can be demonstrated in an isolated perfused liver ; the detailed mechanism of the synthesis is unknown. The formation of liver glycogen in the intact animal is rapid, sugar feeding appreciably increasing the glycogen store. This is the reason for the beneficial effect of sugar ingested before a football match or athletic contest. A well-fed man would have about 200 g. of glycogen in his liver.

Glycogenolysis in the Liver

The liberation of glucose from glycogen in the liver or tissues is called *glycogenolysis*. This takes place under the influence of the enzyme *phosphorylase*, formerly called *glycogenase* (optimum pH 7·2). The product of the reaction is not maltose, as with amylases, but glucose (see p. 225). The action of the enzyme *in vivo* must be controlled in some way, for upon the death of the animal the whole of the liver glycogen is converted to glucose in about two hours. In the living animal there may be large amounts of glycogen in the liver, even in the fasting state. There are two main agents concerned with the control of glycogenolysis, the hormones adrenaline, of the suprarenal glands, and insulin of the pancreas.

Adrenaline stimulates glycogenolysis whether it is injected, or liberated physiologically from the suprarenal glands. The amount required to cause detectable acceleration is extremely small. Now it is well known that adrenaline is released from the suprarenals under a variety of stimuli, such as physical exercise, cold, pain, anger, fear, asphyxia (and therefore hæmorrhage) and certain anæsthetics ; all these stimuli may cause accelerated glycogenolysis, even to the extent of producing *hyperglycæmia* (blood sugar above normal level) and glycosuria. The stimulus evoking adrenaline secretion for the maintenance of blood sugar level in ordinary circumstances is *hypoglycæmia* (blood sugar below normal).

Insulin is an antagonist to adrenaline and inhibits glycogenolysis. If the insulin supply to the liver be cut off, *e.g.*, by pancreatectomy, the blood sugar rapidly rises, with a corresponding fall in liver glycogen.

Since administration of a large amount of glucose at this stage causes a rise in liver glycogen, it is concluded that insulin checks glycogenolysis rather than stimulates glycogenesis. If insulin be injected simultaneously with adrenaline into rabbits, there is no decrease in liver glycogen as there is with adrenaline alone. If an excessive liberation of sugar by adrenaline is checked by subsequent secretion of insulin, it is probable that some of the excess blood sugar is resynthesised to glycogen in the liver.

Now let us see how the sugar released from the liver glycogen is utilised. Cori and Cori studied the utilisation of glucose in starved rats and found that of the administered glucose 18% was deposited as liver glycogen, 25% as muscle glycogen and 44% oxidised. (The remaining 13% would be largely accounted for as losses, owing to technical difficulties.) While these figures cannot be taken as applying to man they provide some indication.

Glycogenesis in Muscle

The muscles are constantly removing sugar from the blood, and the amount is a reflection of the activity of the muscles, since their work is done mainly at the expense of carbohydrate. Prolonged muscular exertion diminishes the liver glycogen. Ingestion of any food which gives rise to glucose results in glycogenesis, not only in the liver, but also in the muscles. The latter process is enhanced by insulin. In experiments of Cori and Cori just quoted, the administration of insulin along with the glucose resulted in utilisation as follows: liver 6%, muscles 36%, oxidation 49%, showing that muscle glycogen formation increased at the expense of liver glycogen. In a depancreatised (eviscerated) animal Dale and his collaborators showed that storage of glycogen in muscle after injection of glucose can only be demonstrated if insulin is also given. The action of insulin in the muscles appears to be stimulation of glycogenesis ; there is no evidence of inhibition of glycogenolysis. Glycogenesis in muscle must be considered from two aspects—glycogenesis from blood sugar and glycogenesis from lactic acid formed from glycogen breakdown during muscular activity (p. 237). The latter process seems to be independent of insulin and takes place normally in depancreatised animals. A well-nourished man of 11 st. (70 kg.) would have about 350 g. of glycogen in his muscles.

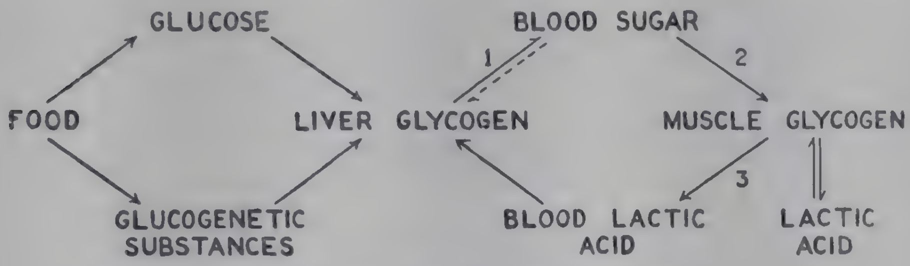
Glycogenolysis in Muscle

The details of the utilisation of glycogen in the muscles must be deferred to a later section. For the moment it is sufficient that in the process of muscular contraction glycogen is broken down to lactic acid, of which about four-fifths is subsequently resynthesised into glycogen, the remaining fifth being oxidised. Some lactic acid, however, may diffuse into the blood, particularly if the muscles contract repeatedly without full time for recovery. This lactic acid is carried to the liver and is resynthesised to glycogen again. As in the liver, adrenaline accelerates glycogenolysis in muscle.

The processes involved in glycogenesis and glycogenolysis are catalysed by phosphorylase. This enzyme, which occurs in many tissues including liver, muscle, heart and brain, can convert glycogen in the presence of phosphate ions into glucose-l-phosphate (*Cori ester*). The reaction is reversible and glycogen can be synthesised from Cori ester by the same enzyme. The phosphorylase of muscle requires adenylic acid as co-enzyme.

Phosphorylases are also widely distributed in plant tissues, *e.g.*, potato, pea and bean. Plant phosphorylases synthesise and hydrolyse starch but have no action on glycogen. Muscle phosphorylase, although it synthesises glycogen *in vivo*, synthesises starch *in vitro*.

The foregoing may be summarised diagrammatically thus :—



ADRENALINE accelerates reactions 1 and 3.
INSULIN accelerates reaction 2.
INSULIN retards reaction 1.

Oxidation of Glucose

Referring back to p. 223, it will be seen that glycogenesis only accounts for about half the absorbed glucose. We must now look to the fate of the remainder, the glucose which is to be oxidised, chiefly in the muscles. In well-fed animals some sugar may be transformed into fat ; this, however, we can neglect for the moment. The difference in oxidation, due to the presence or absence of insulin, can be revealed by studying the *Respiratory Quotient* (R.Q.) of the animal or tissue, that is, the ratio $\frac{\text{Volume of } CO_2 \text{ produced}}{\text{Volume of } O_2 \text{ used}}$.

The R.Q. for the oxidation of fat is about 0·7 and for carbohydrate 1·0. By measuring the urinary nitrogen, the protein metabolised can be found, and the corresponding CO_2 and O_2 deducted, so as to give the *non-protein R.Q.*, which gives the proportions of fat and carbohydrate oxidised (see p. 374). In starvation the non-protein R.Q. will be near that of fat, since carbohydrate oxidation is minimal. If now carbohydrates are given, the quotient in a normal animal will rise, since carbohydrate is being oxidised. But in a depancreatised (*i.e.*, insulin-free) animal the quotient is about 0·7, in spite of there being hyperglycæmia, due to unchecked liver glycogenolysis ; further, giving glucose does *not* raise the quotient, which is only raised if insulin is given with the glucose. The effect can even be demonstrated in isolated tissues. Kidney and muscle tissues of depancreatised dogs have R.Q.'s of about 0·7, which are not raised by addition of glucose ; the same addition to normal tissues raises the R.Q. It must be concluded that lack of insulin diminishes the utilisation of carbohydrate. It also causes diminution in the storage of carbohydrate as glycogen in the muscles (p. 224). Both these effects would be achieved if insulin catalysed the conversion of glucose into some intermediate common to both processes, thereby increasing the rate of entry of glucose from the blood into the muscle cells. Lack of insulin would thus, by delaying the removal of glucose from the blood, diminish the utilisation and storage of carbohydrate in muscle. The phosphorylated hexoses (pp. 225, 237) would be suitable intermediates for such a *rôle* and there is evidence that insulin increases the rate of formation of the phosphorylated hexoses. It is probable that this catalytic influence upon phosphorylation is the fundamental action of insulin.*

Regulation of Carbohydrate Utilisation by Insulin and Adrenaline

Increased Supply. If the supply of blood sugar is suddenly increased, as by intravenous injection or ingestion of a large amount of sugar, the resulting hyperglycæmia will be lowered at a rate dependent upon the amount of insulin secreted, since insulin accelerates muscle glycogenesis and sugar oxidation, *i.e.*, reactions which remove sugar from the blood. If the secretion of insulin is deficient, the hyperglycæmia will persist for a long time, and may result in glycosuria ; in a healthy man the raised blood sugar will be rapidly reduced to the normal level within two hours.

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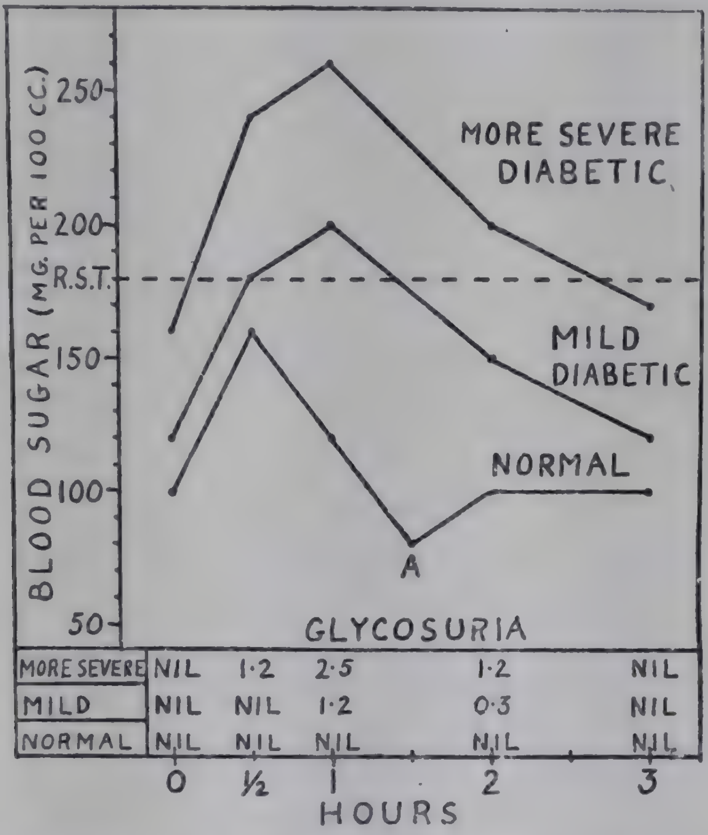
* An alternative explanation of the action of insulin, which has some adherents, is that insulin suppresses excessive formation of sugar from fat, rather than ensuring utilisation of carbohydrate. According to this view, in the absence of insulin so much sugar is formed from fat that the animal's capacity for using sugar is exceeded, hence the hyperglycæmia. (See Ref. 100.)

This provides a clinically convenient method of assessing the efficiency of insulin secretion (Fig. 19).

The curves show the effect of ingestion (after fasting) of 50 g. glucose by a healthy man and two diabetic subjects. The curves of the diabetics rise above the renal threshold and so glycosuria occurs; they also take longer to return to normal. A healthy subject often shows a slight hypoglycæmia at **A**. This represents an outpouring of insulin in excess of that required, so that if a second dose of glucose be given after the blood sugar has regained its original level, the resulting hyperglycæmia is not as great as before.



GLYCOSURIA	0	½	1	2	3
MORE SEVERE	NIL	1·2	2·5	1·2	NIL
MILD	NIL	NIL	1·2	0·3	NIL
NORMAL	NIL	NIL	NIL	NIL	NIL

FIG. 19. Typical blood sugar curves of normal, mild diabetic and more severe diabetic adults after ingestion of 50 g. glucose. R.S.T. = average normal renal sugar threshold (180 mg.). (After curves and data by Harrison, Ref. 3.)

Decreased Supply. If the sugar supply is cut off, as in starvation after the glycogen reserves have been exhausted, the R.Q. is low, indicating that fat and to some extent protein are being utilised almost to the exclusion of carbohydrate. Presumably normal insulin secretion is reduced. If exercise is taken the R.Q. does not rise appreciably, indicating that fat provides the necessary energy for the resynthesis of the lactic acid from the muscle glycogen, for muscle glycogen does not break down to provide blood sugar. If severe hypoglycæmia evokes the secretion of adrenaline, muscle glycogen breaks down to lactic acid, which can be synthesised to glycogen by the liver. The action of insulin and adrenaline can be summarised :—

	Secretion stimulated by	Effect
Insulin . .	Hyperglycæmia	Retards liver glycogenolysis. Accelerates muscle glycogenesis from blood sugar. Accelerates utilisation (but not muscle glycogenolysis).
Adrenaline .	Hypoglycæmia	Accelerates liver glycogenolysis. Accelerates muscle glycogenolysis.

GLYCOSURIA

Glycosuria does not necessarily indicate a condition of diabetes mellitus or even an abnormality of metabolism. Glycosuria may be the consequence of a number of varying conditions, one of which is diabetes mellitus. Glucose is always excreted in small amount. A normal man excretes about 140 mg. in twenty-four hours ; this amount, however, is too small to be revealed by the ordinary urine sugar tests. Sugars other than glucose may be excreted ; these can be regarded more as foreign substances which have been eliminated and will not be considered here. Four typical conditions under which glucose may be excreted are :—

(1) **Alimentary Glycosuria.** Ingestion of abnormal amounts of sugar may result in a hyperglycæmia higher than the renal sugar threshold, so that a transitory glycosuria will ensue.

(2) **Adrenaline Glycosuria.** This form of glycosuria was first observed by Claude Bernard when he punctured the floor of the fourth ventricle in the brains of rabbits, and hence is sometimes referred to as "*Puncture Diabetes.*" In well-fed animals this results in prolonged hyperglycæmia and glycosuria. In starved animals, in which the liver glycogen has been depleted, there is

little response. This hyperglycæmia is due to stimulation of the suprarenals through the sympathetic nervous system promoting secretion of adrenaline, for no result is obtained in the absence of the suprarenals or when the splanchnic nerves are cut. As would be expected from this explanation, the blood lactic acid is also raised (due to increased muscle glycogenolysis). The puncture glycosuria is, in fact, a typical adrenaline hyperglycæmia, which can be imitated by injection of adrenaline (Fig. 20).

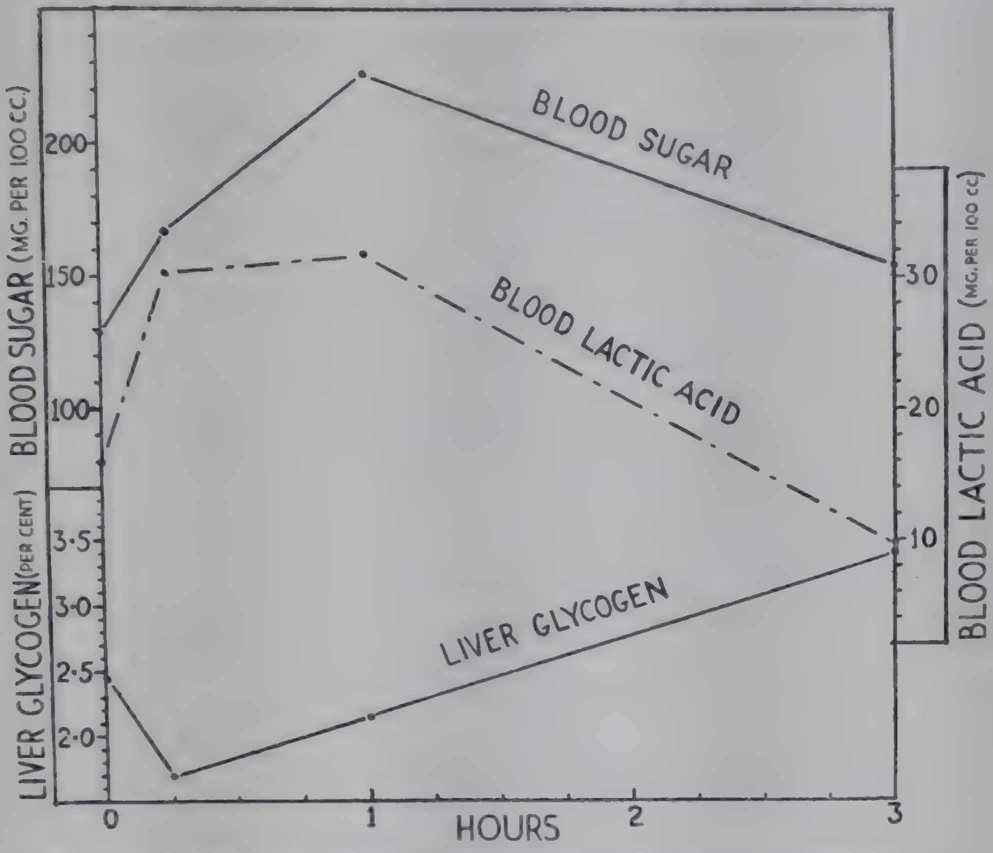


Fig. 20. Effect of subcutaneous injection (at 0 hours) of adrenaline (0·02 mg. per 100 g.) on blood sugar, blood lactic acid and liver glycogen of the rat.

(Data from Cori and Cori, *J. Biol. Chem.*, 1930, **86**, 375.)

In the intact animal glycosuria may be observed after anger, fear, or anxiety, conditions which cause abnormal secretion of adrenaline. Cats, bound in such a way as to cause no pain, exhibit glycosuria, the extent being roughly proportional to their struggling. Emotional glycosuria has frequently been observed in students sitting for an examination. The glycosuria following the use of ether and chloroform as anæsthetics is largely due to hypersecretion of adrenaline.

(3) **Renal Glycosuria.** It was previously stated that the renal sugar threshold was 180 mg. per 100 c.c. This value is, of course, subject to individual variation. Diabetics often have an abnormally high R.S.T.; other individuals, apparently quite healthy, constantly pass small quantities of glucose, due to a low renal threshold which may be below 150 mg. per 100 c.c. Since the condition is apparently harmless, it is referred to as "*Benign Glycosuria*" or "*Diabetes innocens.*" Note that in this form of glycosuria there is no hyperglycæmia.

A severe form of this condition can be induced artificially by poisoning the kidney with phlorrhizin, which prevents reabsorption of glucose from the tubules. The absence of a renal sugar threshold means that there will be a continuous loss of glucose and the blood sugar level will be abnormally low. This hypoglycæmia will evoke the secretion of adrenaline, which will cause accelerated liver glycogenolysis. When the liver glycogen is exhausted, glucose will be formed from protein of the tissues in an endeavour to maintain the blood sugar level. The tissues of the animal will, therefore, waste, and an analysis of the urine, while fasting, will show that the ratio $\frac{\text{Glucose excreted}}{\text{N excreted}}$ (*the G/N ratio*) will be constant. Another consequence of the lack of carbohydrate will be impaired fat metabolism (p. 254), since carbohydrate oxidation is essential for complete oxidation of fat. Therefore there will be ketosis and ketonuria. This condition is not relieved by giving insulin.

The phlorrhizinised animal has been particularly useful for investigating the glucogenetic properties of substances, *e.g.*, amino-acids (p. 272).

(4) **Diabetes Mellitus.** In 1889 von Mering and Minkowski, in the course of experiments in relation to fat metabolism, removed the pancreas of a dog. The result was the appearance of signs identical with those of the incurable condition known as *diabetes mellitus*. Although this condition was recognised as a disordered carbohydrate metabolism, there was at that time no suspicion that the pancreas was concerned with carbohydrate metabolism in any other way than providing amylase in pancreatic juice. It was soon shown that the diabetic symptoms of pancreatectomy

could be eliminated if a piece of pancreas were previously grafted to another part of the body, indicating that the essential constituent of the pancreas was not secreted by the pancreatic duct, but directly into the blood, and was what we now call a hormone. Still later it was shown that the hormone was secreted by the islets of Langerhans, from which its name, insulin, is derived. There were many attempts to prepare a pancreatic extract containing active insulin, but it was not until 1922 that the knowledge gained by the previous failures was crystallised into success by Banting and Best in Macleod's laboratory in Toronto. These workers showed that the fundamental difficulty of obtaining an active extract from the pancreas was that trypsin destroyed insulin very rapidly. They overcame this by ligating the pancreatic duct in dogs and waiting until the enzyme-secreting cells of the pancreas had atrophied from disuse (about ten weeks); the dogs were then killed and from the pancreas an extract was made, which was found to contain active insulin. Insulin is now obtained from slaughter-house cattle by freezing the pancreas immediately on removal from the animal and not allowing the temperature to rise above 0° C. until the insulin has been separated from trypsin (p. 335). Insulin is now available as a crystalline protein (p. 335). The active extract obtained by Banting and Best relieved the symptoms of diabetes mellitus, which is a condition brought about by lack of insulin. The signs of biochemical interest in severe diabetes mellitus and pancreatectomy are :—

(1) *Accelerated Glycogenolysis.* Absence of insulin causes unrestrained liver glycogenolysis, but the glucose formed cannot be used for muscle glycogenesis. Therefore the blood sugar rises.

(2) *Hyperglycæmia.* There is a high fasting blood sugar level with a large and prolonged hyperglycæmia after meals (Fig. 19).

(3) *Glycosuria.* The hyperglycæmia is so great that there is considerable glycosuria. In severe cases the urine may contain 5–10% glucose, and ingested glucose may be excreted almost quantitatively. This is not due to a lowered renal threshold. Many diabetics, in fact, show a high renal threshold.

(4) *Fat Mobilised.* In consequence of the failure to utilise carbohydrate, fats will be utilised to provide energy. The transport of fat in the blood leads to a high blood fat value. The concentration may be so high that the fat separates from the

blood on standing like cream from milk. Values of 20% have been observed.

(5) *Low Respiratory Quotient.* The R.Q. is about 0·7. This would not be attained by excessive utilisation of fat alone, for there is always some protein metabolism (R.Q. 0·81). The value is reduced to 0·7, owing to the conversion of amino-acids to sugar in a vain attempt to build up a glycogen store in the liver. This process is going on continually, for sugar is always being excreted, so that there will be wasting of the tissues.

(6) *Breakdown of Tissue Proteins.* Under fasting conditions, therefore, the G/N ratio will be constant (about 3·6 in man). If protein is taken, there will be no change in this ratio.

(7) *Ketosis.* Failure to utilise carbohydrate means incomplete combustion of fat, and consequently acetone bodies increase in the blood. The acidity of these requires neutralisation, which may deplete the fixed alkali reserve and interfere with respiration.

(8) *Ketonuria* accompanies the ketosis. The urine, besides containing acetone bodies, will be abnormally acid and have a high ammonia content.

All these abnormal signs disappear on administration of insulin, but only temporarily. There are unfortunately two undesirable features in the treatment of diabetics in this way. The first is that insulin must be injected subcutaneously, since, being a protein, it is hydrolysed in the alimentary canal if given orally. So far, no one has succeeded in breaking down insulin without destroying its activity. The best attempt at obviating the unpleasantness of repeated injections has been to reduce the number by using a sparingly soluble compound of insulin with protamine, which can be introduced in larger doses, since its action is delayed. The hypoglycæmic action of insulin or protamine-insulin is also more prolonged if a small amount of a zinc salt is present. The second disadvantage of insulin is that an overdose may be as harmful as a lack. Excess of insulin causes hypoglycæmia. If this is not quickly relieved by injection or ingestion of an appropriate amount of glucose, the blood sugar falls, accompanied by intense fatigue and sweating, with delirium, coma and death if the level falls below 40 mg. per 100 c.c. In a normal man injection of a moderate dose of insulin causes hypoglycæmia, which is soon relieved by liver glycogenolysis. Excess of insulin is dangerous in the diabetic because he has no liver glycogen to mobilise.

This means that the dosage of insulin in diabetes must be planned with great accuracy.

Whilst the cause of diabetes mellitus is usually an *actual* deficiency of insulin, the condition may arise from abnormal function of other endocrine glands. The blood sugar level is maintained by a balance of the actions of several hormones, insulin tending to depress it, adrenaline, anterior pituitary hormones, corticosterone, and thyroxine, either directly or indirectly, tending to raise it. If this balance is upset, *e.g.*, by hypersecretion of the pituitary diabetogenic hormone without a corresponding increase in insulin secretion a *relative* deficiency of insulin would result, having the same effect as an actual deficiency and giving rise to the condition of diabetes mellitus.

A pathological condition of hypersecretion of insulin, *hyperinsulinism*, exhibiting hypoglycæmia, is known clinically.

The Anterior Pituitary Hormone. If the anterior pituitary gland be extirpated, hypoglycæmia follows fasting. Injection of extracts of the gland causes hyperglycæmia and glycosuria. This suggests that the anterior pituitary in some way increases blood sugar. Animals from which the gland is removed are abnormally sensitive to insulin, but if the pancreas is also removed they only show mild diabetic symptoms and live for many months. (Removal of the pancreas only is rapidly fatal.)

Adrenalectomy also reduces the intensity of the signs of diabetes in pancreatectomised animals. The hyperglycæmia and glycosuria are restored by administration of extracts of adrenal cortex or corticosterone (see p. 333).

CARBOHYDRATE UTILISATION IN TISSUES OTHER THAN SKELETAL MUSCLE

So far we have considered only the utilisation of carbohydrate in skeletal muscle. Not all carbohydrate is utilised alike.

Heart Muscle. The metabolism of heart muscle differs from that of skeletal muscle. Heart muscle utilises lactic acid in preference to glucose. A dog's heart takes up about three times more lactic acid than glucose from the blood. Adrenaline has little effect on heart glycogen. During starvation and in pancreatectomised animals heart glycogen increases. The heart, being a vital organ, is protected at the expense of other organs.

Brain. Brain, judging by its R.Q., uses mainly carbohydrate. It forms lactic acid from glucose, and probably oxidises lactic acid. Since there is very little glycogen in brain, it is probable that the lactic acid is formed directly from blood sugar.

The Conversion of Sugar to Lactic Acid. Sugar is apparently readily converted into lactic acid in red blood corpuscles. The lactic acid in the blood of a subject at rest is believed to be formed in this way, lactic acid only diffusing from the muscle during exercise. The disappearance of glucose from shed blood (glycolysis) is due to conversion into lactic acid. This process is slow, but of sufficient magnitude to make it necessary to prevent the reaction by adding suitable preservatives if blood has to be kept for subsequent blood sugar analysis.

THE CHEMICAL CHANGES INVOLVED IN GLYCOGENOLYSIS IN MUSCLE

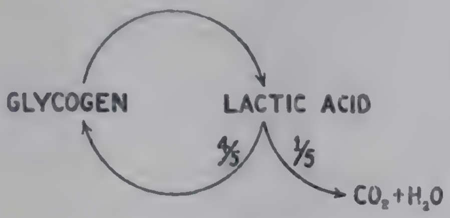
The classical experiments of Fletcher and Hopkins on frog muscles revealed that, by stimulating a muscle to make it contract until fatigued, the lactic acid content was increased from 0·015% to 0·16%. Since the higher concentration indicated the point of complete exhaustion and lack of response to stimulus, it was called the *Fatigue Maximum.* On resting, the lactic acid disappeared at a rate proportional to the availability of oxygen, the muscle at the same time recovering its contractility. If, however, oxygen was withheld, the lactic acid did not disappear and the muscle became irreversibly contracted (*rigor mortis*) and died. These changes, however, were not peculiar to the contraction process, for lactic acid formed slowly in the same way if the muscle remained at rest but deprived of oxygen. It is now agreed that muscular contraction is a special aspect of a series of changes continually occurring in the resting muscle. Subsequent investigations, in which Embden and Meyerhof were pre-eminent, have revealed that the lactic acid is produced as the result of many reactions.

Studies of the respiration (O_2 consumption and CO_2 production) of a muscle show that the net result is the conversion of glycogen to CO_2 and H_2O. The formation of lactic acid is an intermediate stage which proceeds in the absence of oxygen. The oxygen is required for removal of lactic acid. The reactions can be represented:—

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow 2n\ CH_3 . CHOH . COOH$$

$$CH_3 . CHOH . COOH + 3O_2 \rightarrow 3CO_2 + 3H_2O.$$

Actually, however, the oxygen used in the removal of lactic acid from a muscle is only about one-fifth of that which would be expected from this equation. The remaining four-fifths of the lactic acid is resynthesised to glycogen again—a process requiring no oxygen. This can be expressed :—



It must, however, be recognised that the oxygen consumption figures do not prove that lactic acid is oxidised, but only that some compounds with similar empirical composition are oxidised. The purpose of the oxidation to CO_2 and H_2O is to provide energy for the resynthesis of lactic acid to glycogen. It may be that all the lactic acid is resynthesised and some other substance oxidised. In carbohydrate starvation the R.Q. of muscle is low, suggesting that fat oxidation takes place, to provide energy for resynthesis of glycogen.

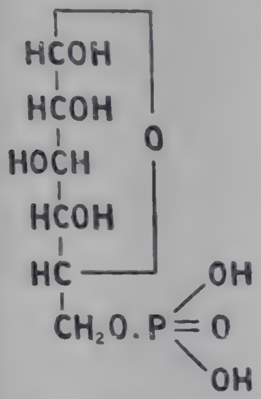
The above scheme applies when the body is at rest or gently exercised. With moderate (walking at 4 m.p.h.) or severe exercise the lactic acid will have an additional path. In contraction of a muscle the change from glycogen to lactic acid is greatly accelerated compared with the rate in a resting muscle, and therefore lactic acid will have to be removed at a correspondingly greater rate. We have already seen that the rate of removal of lactic acid is dependent upon availability of oxygen. In the intact animal the oxygen is, of course, provided by the blood. Now muscle has a rich capillary supply, so that in mild exercise the muscle will get sufficient oxygen to prevent an accumulation of lactic acid ; but as the exercise gets more severe the oxygen supply will be progressively less able to cope with the lactic acid production, even though the respiration is increased. In severe exercise so much lactic acid may be formed that the muscles will take some time to recover, *i.e.*, remove the accumulated lactic acid by oxidation. The muscles have gone into an " *oxygen debt*," and there will be increased pulmonary ventilation until the debt has been wiped off. We must all have observed that after running five minutes it takes about ten minutes to " get our breath." Many medical students have the opportunity of recording their oxygen debt after pedalling on a bicycle ergometer " all out " for two minutes, and finding that it may take half an hour to repay.

The "oxygen debt" of complete exhaustion is about 15 litres, corresponding to an accumulation of about 90 g. of lactic acid.

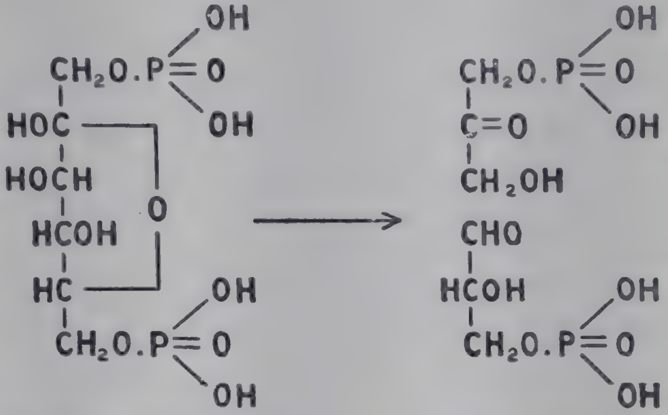
Whenever there is an oxygen debt, some of the accumulated lactic acid diffuses out of the muscles into the blood, and the lactic acid content of the blood rises according to the severity of the exercise. Even at rest there is some lactic acid (5–20 mg. per cent.) in blood, but this is derived from glucose in the erythrocytes (p. 234). A quick walk may raise the value to 50 mg. per cent., and severe exercise even to 200 mg. per cent. Part of this lactic acid is absorbed by the liver and converted into glycogen (p. 224), and a small amount may be excreted by the kidney. Thus in severe exercise lactic acid, in addition to being resynthesised to muscle glycogen or oxidised, may form liver glycogen or be lost in the urine.

It is customary to speak of the liberation of lactic acid (and phosphoric acid) without adding that it is neutralised by some suitable base. Thus in the muscle any small accumulation of lactic acid could be neutralised by muscle proteins or bicarbonate. In severe exercise when lactic acid diffuses out of the muscle, it will be neutralised by the blood bicarbonate, the CO_2 formed being a stimulus to the respiratory centre for increased pulmonary ventilation. Under these conditions the blood pH is lowered very slightly. A muscle only gets appreciably acid when severely exercised, and actually in the early stages of activity gets more alkaline, due to liberation of the strong base creatine (see p. 238).

A study of the conditions affecting the fatigue maximum of muscle showed that this point was reached before all the muscle glycogen was exhausted. The glycogen could, however, be utilised completely if sodium phosphate were added to the liquid bathing the muscle, the fatigue maximum then rising even to 0·83%. This indicated an important *rôle* for phosphate. Inorganic phosphate is, in fact, liberated from a muscle during contraction along with lactic acid. From this point most of our further knowledge of the intermediate chemical changes has been derived from study of cell-free muscle extracts, since the intermediate products cannot be isolated from the muscle. Even under these conditions investigation is difficult, for the intermediate products are very quickly converted to the next stage. The essential intermediates in the process are a hexosemonophosphate (*lactacidogen*) and a hexosediphosphate. The exact chemical nature of these compounds is not certain. The monophosphate is probably an equilibrium mixture of glucose and fructose 6-monophosphoric acid. (Both pure substances quickly form an equilibrium mixture of about 70% glucose ester and 30% fructose ester.) The hexosediphosphate is probably a fructose diphosphate and breaks down, giving a series of 3-carbon phosphates which we can call " triose phosphate."



Glucose-6-Monophosphoric Acid



Fructose-1, 6-Diphosphoric Acid

Triose Phosphoric Acids

The stages in the conversion of glycogen to lactic acid are probably :—

(1) Glycogen→Hexose.
(2) Hexose + Phosphoric Acid →Hexose Monophosphate.
(3) Hexose Monophosphate + Phosphoric Acid →Hexose Diphosphate.
(4) Hexose Diphosphate→2 Mols. " Triose Phosphate."
(5) " Triose Phosphate "→Lactic Acid + Phosphoric Acid.

The " triose phosphate " stages actually involve five different 3-carbon compounds.*

These stages do not require oxygen or produce CO_2. The resynthesis of glycogen is probably a reversal of these changes.

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* These stages are :—

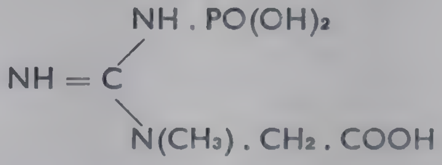
(*a*) Hexose Diphosphate → Glycerose Phosphate + Dihydroxyacetone Phosphate.
(*b*) Glycerose Phosphate + Dihydroxyacetone Phosphate → Glyceric Acid Phosphate + Glycerol Phosphate.
(*c*) Glyceric Acid Phosphate → Pyruvic Acid + Phosphoric Acid.
(*d*) Pyruvic Acid + Glycerol Phosphate → Lactic Acid + Glycerose Phosphate.
(*e*) 2 Glycerose Phosphate → Glyceric Acid Phosphate + Glycerol Phosphate.
(*f*) Hence to Lactic Acid by (*c*) and (*d*), and so on.

The formation of lactic acid is exothermic, *i.e.*, liberates heat or energy. The resynthesis is endothermic, *i.e.*, requires energy. The energy is provided by oxidation of the equivalent of one-fifth of the lactic acid. The details of this oxidation are not of great significance in muscle contraction; all that is required is the provision of energy by an exothermic reaction.

There seems little doubt that the lactic acid formation is intimately associated with the contraction of muscle, for the tension developed is proportional to the amount of lactic acid formed. Further, the lactic acid content of different fatigued muscles is a rough measure of their activity; in the intact animal the amount of lactic acid escaping into the blood corresponds with the severity of muscular exercise. It was thought that the contraction of a muscle was developed as a result of the formation of lactic acid from glycogen, the heat produced thereby providing the energy for the contraction; but recently extremely delicate thermopiles devised by A. V. Hill have shown that the lactic acid does not appear until after the contraction and relaxation are completed; further, Lundsgaard has shown that if a muscle be poisoned with iodoacetic acid, it can contract and relax without any lactic acid being formed, so that the cause of the contraction must be sought in other reactions.

There are two other compounds occurring in muscle which are concerned in the contraction process: *creatine phosphoric acid* (*phosphagen*) and *adenyl pyrophosphate.*

Phosphagen. Creatine phosphoric acid,



is a readily diffusible substance and must be prevented from diffusing from the muscle in some way, probably by combination with some indiffusible substance. This compound is called **phosphagen.** During contraction creatine phosphoric acid breaks down to creatine and phosphoric acid and is resynthesised during recovery. But the reactions do not run parallel with the glycogen-lactic acid mechanism, for in the normal muscle under anaerobic conditions the phosphoric acid produced from creatine bears no relation to the lactic acid formation or muscle tension. It is formed more quickly at the beginning of a series of contractions, and may almost stop during fatigue while lactic acid is still increasing. On the other hand, in muscles poisoned with sodium iodoacetate the creatine phosphoric acid breakdown is proportional to the tension (it will be remembered that lactic acid is not formed under these conditions). Under aerobic conditions creatine and phosphoric acid are quickly resynthesised, although a small resynthesis can occur anaerobically. It has not been possible to determine whether breakdown of creatine phosphate causes the contraction or is a consequence of it.

Adenyl Pyrophosphate. This substance is the pyrophosphate * of adenylic acid (p. 116). Since adenylic acid contains one phosphoric acid group, adenyl pyrophosphate is, in effect, a triphosphate (adenosine triphosphate, often abbreviated A.T.P.). Adenyl pyrophosphate is a necessary co-enzyme for the conversion of glycogen to lactic acid, and acts as a phosphoric acid " carrier " transferring phosphoric acid to hexose, and subsequently removing it from " triose phosphates." This can be represented :—

Adenyl Pyrophosphate + Glycogen →
Hexosephosphate + Adenylic Acid.
Adenylic Acid + " Triose Phosphate " →
Adenyl Pyrophosphate + " Triose."

Adenyl pyrophosphate is also intimately connected with creatine phosphate. These compounds take part in reversible reactions, which can be summarised :—

Adenyl Pyrophosphate + Creatine ⇌
Creatine Phosphate + Adenylic Acid.

(Adenosine diphosphate is an intermediate product in this scheme.)

We have, then, in the muscle a complex mechanism for degrading glycogen to lactic acid through phosphorylated hexoses and " trioses," with adenylic acid and creatine phosphate acting as phosphate " carriers." A complete scheme † of these reactions

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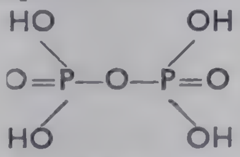
* The structure of pyrophosphoric acid is



† Such a scheme showing the energy values of the various reactions is given in " Annual Reviews of Biochemistry," 1937. See also Ref. 100.

is beyond our scope. The ultimate effect is a system which "discharges" to provide energy without oxidation; this system is subsequently "recharged" by energy derived by oxidation. It is not yet certain whether the initiation of the "discharge" is a chemical reaction such as the breakdown of adenyl pyrophosphate, or a physical mechanism.

It may perhaps be added that adenylic acid in muscle can also undergo deamination to ammonia and inosinic acid in the subsequent resynthesis, and that there is probably another path of lactic acid formation through methyl glyoxal.

It must be remembered that the experimental basis of the foregoing is very largely founded on the behaviour of skeletal muscles in the frog. What evidence there is suggests that the behaviour of other voluntary and cardiac muscles is qualitatively similar, although some species contain *arginine phosphoric acid* in place of creatine phosphoric acid. Quantitatively, however, muscles may differ considerably. Heart muscle, for example, contains relatively little creatine phosphoric acid, and cannot be stimulated to such great activity as ordinary skeletal muscle. As to plain muscle, we know very little except that the creatine content is extremely small. Although many reactions occurring in muscle have been discovered, it is certain that we are very far from the complete story.

CONVERSION OF CARBOHYDRATE INTO FAT

There is ample evidence of the ready conversion of sugar into fat. See pp. 295, 296.

CHAPTER XVIII

UTILISATION OF LIPIDES (1, 4, 6, 8, 21, 36, 45, 86, 89)

DIGESTION

Lipides form part of the diet of all animals. In man the amount ingested varies considerably with locality. In this country lipides compose between one-fifth and one-seventh of the solids in our food, and are chiefly in the form of triglycerides. The lipides are hydrolysed during digestion by lipases and phosphatases to fatty acids, glycerol, phosphoric acid and other products. The lipases appear to have no specificity, such as the proteases and carbohydrases, and attack all true fats. Fat digestion is peculiar in that, although completely hydrolysed in the intestine, fats are resynthesised at the point of absorption.

Gastric Digestion. There is no digestion of fat in the mouth. The first lipase is encountered in the stomach. It has been stated that a lipase—**gastric lipase**—is secreted in the gastric juice and is quite distinct from pancreatic lipase, which might reach the stomach by regurgitation. Gastric lipase, however, is very easily inhibited or destroyed by the acidity of the gastric juice. Ordinarily there can be little fat splitting in the stomach, not only on this account, but also because there are no means by which fat can be emulsified. Appreciable hydrolysis probably only occurs when the fat is already emulsified as in milk or egg-yolk, or under conditions in which the normal acidity of the stomach is neutralised or reduced sufficiently to prevent the destruction of gastric lipase. If the HCl secretion in the stomach is deficient, digestion by gastric lipase may be of value, since in the absence of acid one of the normal stimuli of pancreatic secretion will be lacking.

If the emptying of the stomach be delayed, as by excess of fat in the food, regurgitation of the duodenal contents, containing bile and pancreatic juice, may occur and cause some fat hydrolysis. Such digestion would be largely due to pancreatic lipase.

Intestinal Digestion. Ingested fat may be hydrolysed in the small intestine by the lipase, **steapsin,** of pancreatic juice. In pure pancreatic juice steapsin is relatively inactive, but it is activated by several substances, *e.g.*, bile or Ca salts. In addition to lipase, bile is essential for the normal digestion and absorption of fat. The absence of bile, as in obstruction of the bile duct, largely prevents the absorption of fat; but if the pancreatic duct be obstructed, 50% of ingested fat may be absorbed, due either to the presence of lipases in the succus entericus or to absorption of unhydrolysed fat. (Normally, practically all ingested fat is absorbed.) Bile owes its importance to several properties.

(1) Bile contains bile salts, which are very effective in lowering surface tension; consequently bile is a good *emulsifying agent.*

(2) It also has considerable *solvent action* on lipides and fatty acids, thus facilitating the action of lipase.

(3) Bile salts are *activators* of pancreatic lipase.

(4) Bile salts are reabsorbed from the intestine and pass back to the liver, stimulating further secretion of bile—*cholagogue action.*

(5) Bile helps to *neutralise* the HCl of gastric contents.

But by far the most important function of bile is to facilitate the *absorption* of fatty acid (p. 224).

Soluble soaps are also effective in promoting emulsification, and were at one time considered of even greater importance than bile in this respect. Since soaps of the common fatty acids do not exist below pH 8, they could only be effective in the immediate neighbourhood of the pancreatic duct where the reaction may be alkaline (about pH 8). They certainly cannot exist in the greater part of the intestine where the reaction is acid.

Another factor which may assist the emulsification of fat—at any rate in the early stages of digestion—is the presence of soluble proteins of pancreatic and intestinal juice.

In addition to the lipase of pancreatic juice, there are lipases in intestinal juice, which can act upon any fat left unaffected by pancreatic lipase. These enzymes can probably digest fat completely in the absence of pancreatic lipase; the reason for the poor utilisation of fat under these conditions is probably incomplete absorption, due to delayed digestion rather than incomplete digestion, since the fat found in the fæces is "split" or hydrolysed.

ABSORPTION (39, 89, 97)

The manner in which fat is absorbed through the intestinal epithelium has long been disputed. It is certain that after absorption fat droplets appear inside the epithelial cells. It was at first supposed that fat actually passed through the cell walls in the form of minute fine particles of fat, a view largely supported by histological experiments showing the appearance of ingested dyed fats inside the epithelial cells. However, the recognition of the presence of active fat splitting enzymes in the small intestine and the demonstration that dyed fat can pass into the epithelial cells without the dye showed that at least some fat was completely hydrolysed before absorption. So convincing were these experiments at the time that it became generally accepted that complete lipolysis was an essential preliminary to absorption (*Lipolytic Hypothesis*), until the recent reinvestigations of Frazer and his collaborators clearly showed that fat may be absorbed both in the hydrolysed and unhydrolysed condition (*Partition Hypothesis*).

Lipolytic Hypothesis. This suggests that neutral fat is completely hydrolysed in the lumen of the gut. The resultant fatty acids and glycerol are absorbed into the cells of the intestinal epithelium where they are resynthesised into fat which then passes up the lacteals and the thoracic duct into the systemic circulation.

The passage of the soluble glycerol into the epithelial cells presents no difficulties, but the passage of insoluble fatty acids requires explanation. Fatty acids cannot be regarded as soluble soaps, as was customary in the past, for the pH in the region of absorption is practically never above pH 7 and usually definitely acid. The explanation appears to lie in the peculiar properties of bile salts. Their effect is well shown in the following experiment on four young dogs of the same litter narcotised with chloralose. After tying the common bile duct, the small intestine was washed out with saline, tied at the ileocæcal valve and filled with the solutions given in the table.

Solution	Olive Oil found in the Intestine after 24 Hours		Absorption
	As Neutral Fat	As Fatty Acid	
Olive oil + soap emulsion	97·2%	0·9%	None
Olive oil + lipase.	57·6%	43·7%	None
Olive oil + Na taurocholate . . .	96·3%	1·1%	None
Olive oil + lipase + Na taurocholate .	17·7%	7·8%	74·5%

(Calculated from Verzár. Ref. 39, p. 153.)

The animals were killed twenty-four hours later and the contents of the small intestine analysed. The results show the value of Na taurocholate in the absorption of digested fat. Glyco- and tauro-cholic acids are hydrotropic substances which have the power of forming compounds with fatty acids which are not only soluble but *diffusible* even at pH 6·0 (p. 48). This explains the absorption of fatty acid, but raises a quantitative question. To form a hydrotropic compound about 5 g. of bile acid are required for every 1 g. fatty acid. Now a man will not excrete more than 90 g. (= 1,500 c.c. of bile) of bile acid a day, *i.e.*, enough to form a hydrotropic compound with 18 g. of fat. Actually a man would absorb five times this amount. The explanation has been put forward that the bile acid–fatty acid (choleic acid) complex is broken down as soon as it enters the epithelial cell; the bile acid liberated is absorbed at the surface of the epithelial layer, where it dissolves more fatty acid and brings it into the cell.

The resynthesis of fat in the cells of the intestinal epithelium is thought to involve the intermediate formation of phosphatide, the first stage probably being phosphorylation of the glycerol (*i.e.*, formation of glycerophosphate). The evidence includes the observations that absorption of fatty acids is accelerated by glycerol and phosphate, and that the synthesis is inhibited by drugs known to inhibit phosphorylation, such as phlorrhizin and iodoacetic acid. When fat is fed to animals along with radioactive phosphates, it can be shown that in a short time the phospholipides of the intestinal mucosa contain radioactive phosphorus. The mechanism of the conversion of phosphatide into neutral fat is not known. It is practically certain that the conversion is not complete, some phosphatide appearing in the lymph, so that the phosphatide content of the blood is increased during absorption of neutral fat.

The synthetic mechanism is not dependent on ingested glycerol. If fatty acids alone are administered, neutral fat is recovered in the chyle. The glycerol must presumably be obtained from other sources *viâ* the blood.

Extirpation of the suprarenals inhibits fat absorption, which is restored to the normal rate by administration of cortical hormone or large amounts of NaCl. This may be the explanation of the impaired fat absorption in certain pathological conditions (*e.g.*, Addison's disease) involving disturbance of the suprarenal cortex (see p. 334).

Cholesterol, or at any rate the greater part of it, is also involved in synthesis in the epithelial cell—into cholesterol esters of fatty acids. In the absence of fatty acids it is not appreciably absorbed. It is perhaps significant that two molecules of phosphatide could provide fatty acids for one molecule of true fat and one molecule of cholesterol ester. The absorption of cholesterol is curiously selective, several very closely related sterols not being absorbed at all.

Partition Hypothesis. This postulates that some ingested neutral fat is absorbed unhydrolysed and passes by the lymphatic system and thoracic duct into the systemic blood, whereas the fat which is hydrolysed is not resynthesised but passes as glycerol and fatty acid via the portal vein to the liver where it is metabolised. It accounts for a number of experimental findings which are not satisfactorily explained by the lipolytic hypothesis, *e.g.*

(1) Complete inhibition of lipolysis by sodium cetyl sulphate in rats does not prevent triglyceride absorption.
(2) The different behaviour upon absorption of fat and of an equivalent mixture of fatty acids and glycerol. If complete lipolysis occurs before absorption there should be no difference.
(3) If lipase sufficient to ensure complete lipolysis is added to the fat in (2) the results are the same as with the mixture of fatty acids and glycerol, showing that complete hydrolysis had not, in fact, occurred in (2).

The mechanisms involved in the absorption of unhydrolysed fat are very complex involving many physico-chemical considerations. It is essential that the fat be very finely emulsified, the diameter of the droplets being less than $0{\cdot}5\mu$. It would seem that the triple combination fatty acid, bile salt and monoglyceride is necessary for effective emulsification under the conditions which prevail in the small intestine. It has been proved that monoglyceride is formed as an intermediate product during the lipolytic hydrolysis of triglycerides.

It has been shown that very finely emulsified hydrocarbons (liquid paraffin) can be absorbed.

The Passage of the Absorbed Lipides into the Blood

Lipides, whether absorbed as such or resynthesised in the intestinal epithelial cells, enter the lacteals as a milky emulsion called **chyle.*** How this transfer is accomplished has yet to be discovered. Several theories have been postulated, but none are supported by convincing evidence. The possibility of diffusion of finely emulsified lipides through the cells wall has not been excluded.

From the lacteals the chyle passes through the lymphatics of the mesentery to the receptaculum chyli, and is delivered through the thoracic duct into the blood at the junction of the subclavian and jugular veins.

Only about 62% of ingested lipides can be recovered in the thoracic duct. Since normally not more than 2% ingested fat can be traced in fæces, this leaves 36% to be accounted for. According to the partition hypothesis this 36% would represent the fat which has been hydrolysed, absorbed as fatty acid and glycerol, and passed to the liver *viâ* the portal vein.

INTERMEDIARY METABOLISM OF LIPIDES

Lipides in Blood. The fine suspension of lipides from the chyle enters the blood at the jugular vein and thence passes round the body. The resulting lipæmia (increase of lipides in the blood) may last a few hours, the actual time varying with the amount of fat absorbed and activity of the subject. It is usually stated that the maximum lipæmia occurs after four hours ; it cannot always be detected in man. How the lipides leave the blood is not certain. It is improbable that they pass into cells of the fat depôts, liver and other tissues by hydrolysis and resynthesis, since neither blood nor tissue cells possess true lipases.† Since capillary walls and, presumably, the cell walls have a permeability comparable with artificial membranes through which serum lipides can be shown to diffuse without change in composition, it is likely that the lipides pass into the tissue cells unchanged. It is significant that the plasma fat is extremely finely emulsified, a process probably assisted by the passage of blood through the lung capillaries.

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* The lipides in the chyle are in the form of minute droplets, 0·05–1μ diameter, called **chylomicrons**. After a standard meal of 30 g. fat more chylomicrons are observed in the blood of fat subjects than of thin ones.

† There are enzymes present in most tissues which split simple fatty acid esters, phosphatides, and possibly cholesterol esters, but in most instances they will not split the glycerides of ordinary fat. These enzymes have been called lipases, but are more correctly described as esterases.

The next stage is either the deposition of fat in the tissues or its immediate oxidation for energy purposes.

Lipides in the Tissues

Stored Fat. In animals fat may be deposited in many tissues, but by far the most important deposits are in the subcutaneous and retroperitoneal connective tissues. Fat is the chief reserve store of the animal, since very little carbohydrate is stored. A man can only store about 500 g. (*i.e.*, a little more than 1 lb.) of glycogen, but we have only to look at certain of our fellows to see that more fat may be stored. A dog has even been induced to store fat equal to 40% of its body weight.

It must not, however, be thought that this store (depôt) fat is out of reach of general metabolic processes. Experiments with deuterium labelled fat have shown that when such fats were fed to mice about half was deposited in the fat depôts. Since the total amount of depôt fat remained constant this meant that approximately equal amounts of food fat and store fat must have been oxidised, the store fat having been replaced by an equivalent amount of food fat.

Now the store fat is derived not only from the fat in the diet, but also contains fat formed from carbohydrate. The composition of the depôt fat is not the same as that of the food fat. If a saturated fat is fed the depôt fat has a lower melting point and a higher iodine value, while feeding olive oil gives a depôt fat with higher melting point and lower iodine value than the olive oil. An animal's fat does not resemble its food fat, but tends to be characteristic for each species of animal. An analyst would have little difficulty in distinguishing between the fat of beef or mutton. Experimentally we can induce great changes in the depôt fat by interfering with the animal's diet, especially if the depôt is first depleted by starvation. Thus a starved dog fed largely on mutton fat lays down a depôt fat more closely resembling sheep fat than dog fat. Or, if linseed oil be used, the depôt fat is liquid at 0° C. Similarly, fats "labelled" with bromine or deuterium or fats containing fatty acids peculiar to plants (*e.g.*, erucic) can be introduced into the depôt fat. The experiments cited are extreme cases; nevertheless, distinct changes can be produced in animal fats by moderate changes in diet, and not necessarily in the food fat, for body fat can be readily synthesised from carbohydrate. Fat so formed is more highly saturated and has a higher melting-point and is termed "hard" fat. This is of considerable agricultural importance. The hard fats of grazing animals are largely formed from carbohydrate. Feeding on excess oil-cake may appreciably soften the fat. Pigs which are fed on meal made from oily seeds are frequently "finished" by a period of grain feeding to harden the fat. The characteristic composition of the fat of any given species must therefore be partly due to a constant diet.

There are small differences in composition in the store fat in different parts of the body of the same animal.

The "Fat" of Ordinary Tissues. Apart from the obvious store tissues, fat can be found in many other tissues of the body. This fat is not only store fat. Two functions can be distinguished which have led to the terms *constant element* and *variable element*. The *constant element* represents the fat content of the tissue which remains constant during starvation and cannot be reduced without death occurring, *i.e.*, it represents *vital* cellular constituents; the *variable element* represents the fat which disappears during starvation, *i.e.*, the store fat. The former consists mainly of phosphatides and cholesterol; the latter neutral fat. The phosphatides have relatively more unsaturated fatty acids than have the corresponding neutral fats; their function is assumed to be connected with intermediary fat metabolism. The phosphatide content of organs is roughly proportional to their functional activity. In distinction to the store fats, the phosphatides in the tissues are not revealed by histological staining, apparently forming an association with the protoplasm (protein ?) in the cell; they are even difficult to extract with many fat solvents, only being effectively removed by hot alcohol. Thus normal heart muscle histologically appears to contain very little fat; if, however, the muscle cells be damaged, as by phosphorus poisoning or certain bacterial toxins, globules of readily stainable fat appear (*fatty degeneration*). No fat is formed in this process, since chemically there is the same amount of fatty acid in each case; the fat has been revealed by the break-up of the phosphatide-protein association.

As to individual organs, the lipides of the brain, kidney, lung, heart and spleen are not affected by starvation, *i.e.*, their fat is only constant element. This is shown by the fact that there is no appreciable difference in the total fatty acids of these organs in well-fed, normal or starved animals. Figures for the kidney in dogs are 11·1%, 11·9% and 13·4% respectively. Corresponding figures for muscle are 17·6%, 11·3% and 4%, so that muscle must be regarded as a tissue with considerable reserve of fat. The fat content of the liver supports the view that fat is mobilised in the liver as a stage in its utilisation. Normally the percentage of fat in liver is low (3% phosphatide, 1% true fat) with a high iodine value (115–135); during starvation the percentage of true fat rises (up to 20%), while the iodine value falls, indicating that the liver is being flooded with neutral fat (iodine value 35–65). This increase, however, is only transitory and the liver fat falls as the stores are used up.

Liver lipides may be increased under certain abnormal conditions. Feeding cholesterol results in accumulation of fat and cholesterol esters in the liver ("fatty liver"), which may be prevented by feeding with choline, lecithin, methionine or betaine. Substances which prevent this abnormal accumulation of fat in the liver are called **lipotropes** or **lipotropic factors.** Dietary deficiency of lipotropes causes the liver to synthesise extra fat from carbohydrate. Proteins have a small lipotropic effect probably mainly due to their content of methionine.

Lipotropes are not, however, the only factors concerned in the formation of fatty livers. The condition is frequently associated with decreased liver glycogen, *e.g.*, in diabetes mellitus. The accumulation of particular lipides in the liver or other organs is characteristic of certain diseases, *e.g.*, of phospholipides, especially sphingomyelins, in Niemann-Pick disease and of cerebrosides (kerasins) in Gaucher's disease.

Special attention might be drawn to the brain, which does not lose weight during starvation. The words of Leathes are most apt: "*The organ which contains the largest amount of what may be called organised fat can dispense with none of it if it is to live.*"

The term constant element is not a good one, since it is only the amount which is constant. The composition (degree of saturation) can be changed by diet. Cod-liver oil produces phosphatides which are more unsaturated, a "fat-free" diet phosphatides which are more saturated.

We have, then, in the tissues two kinds of fat—the phosphatides and associated lipides, including cholesterol, which are essential cellular constituents, and the neutral reserve fat, which can be used for general purposes. The part played by the former in the life of the cell has yet to be discovered. Some detailed aspects of the utilisation of neutral fats, however, have been revealed by experiment. The ultimate products of the oxidation of fat are CO_2 and H_2O.

Oxidation of Fats

Glycerol. Glycerol is apparently utilised in the same way as carbohydrate. It can give rise to sugar and behaves as an antiketogenic substance (p. 254).

Fatty Acids. The early stages in the utilisation of long chain fatty acids is far from clear. The changes, whatever they are, take place largely if not entirely in the liver. The usually accepted view is that the neutral fat is converted into phosphatide by replacement of one of the fatty acids by a phosphoric acid complex (*e.g.*, phosphoric acid—choline).

The evidence is :—

(1) Phosphatides are universal constituents of all cells.

(2) The increase in blood and liver phosphatide during neutral fat absorption.

(3) Food fat may have some influence on the composition of the phosphatides of the tissues. The phosphatides of hens' eggs may be affected similarly.

(4) There is evidence that the fat of cow's milk is mainly produced from blood phosphatides.

(5) Artom has shown that rats fed on olive oil and radioactive sodium phosphate have radioactive phosphate in the liver phosphatides nine hours later.

The advantages of phosphatides as a preliminary stage of fat utilisation are :—

(*a*) They mix readily with blood plasma and would form a ready means of transport of insoluble fats.

(*b*) They are chemically more reactive, being more easily hydrolysed. (Fats are only hydrolysed by lipases, but not esterases ; phosphatides can be hydrolysed by esterases, which are present in practically all tissues of the body.)

It is uncertain whether the phosphatides are formed in the tissue cells and transported to the liver, or whether fats are carried to the liver and there transformed into phosphatide.

The next stage is that phosphatides formed from fat undergo a process of desaturation, probably in the liver. The evidence upon which this was originally based arose from an attempt to explain the important *rôle* of the liver in fat metabolism.

Leathes found that even the highly unsaturated fats of cod-liver oil and herrings when fed to cats and rats gave liver fats with iodine values 30% higher than the fed fat. Hartley compared the depôt and liver fat of the pig. The depôt fat contained palmitic, stearic and oleic acid $\triangle^{9:10}$ (oleic acid with double bond between ninth and tenth carbon atoms). The liver fat contained palmitic, stearic, oleic acid $\triangle^{12:13}$ (but not $\triangle^{9:10}$) and lineolic acid $\triangle^{9:10,\ 12:13}$, as well as about 10% of arachidonic acid with four double bonds. This suggested the insertion of a double bond between the twelfth and thirteenth carbon atoms of both stearic and oleic acid thus :—

$$\text{Stearic Acid} \longrightarrow \text{Oleic Acid } \triangle^{12:13}$$
$$\text{Oleic Acid } \triangle^{9:10} \longrightarrow \text{Linoleic Acid } \triangle^{9:10,\ 12:13}$$

In recent experiments, however, Turner and Channon have failed to find any trace of the oleic acid $\triangle^{12:13}$.

Recently Schoenheimer and Rittenburg have provided what would appear to be decisive proof of desaturation. They fed mice on fully saturated fatty acids containing a high content of deuterium (heavy hydrogen). Unsaturated fatty acids containing deuterium were subsequently isolated from the depôt fat. It is not certain where this desaturation occurred.

The next step in the katabolism of these unsaturated fatty acids is unknown. In some way fatty acid chains, possibly of reduced length, are presented for oxidation.

β-Oxidation

The mechanism of the oxidation of the fatty acid chains was revealed largely by the classical experiments in which Knoop administered to dogs phenyl derivatives of the fatty acids. Aromatic compounds of this type are not easily oxidised in the animal, so that end products could be recovered from the urine (ordinary fatty acids are oxidised to CO_2 and H_2O). The results are given in the table.

Acid administered	Acid excreted	C atoms lost
Phenyl formic acid, $C_6H_5 . COOH$	$C_6H_5 . COOH$	0
Phenyl acetic acid, $C_6H_5 . CH_2 . COOH$	$C_6H_5 . CH_2 . COOH$	0
Phenyl propionic acid, $C_6H_5 . CH_2 . CH_2 . COOH$	$C_6H_5 . COOH$	2
Phenyl butyric acid, $C_6H_5 . CH_2 . CH_2 . CH_2 . COOH$	$C_6H_5 . CH_2 . COOH$	2
Phenyl valeric acid, $C_6H_5 . CH_2 . CH_2 . CH_2 . CH_2 . COOH$	$C_6H_5 . COOH$	4

As a point of accuracy benzoic and phenylacetic acids are not normally excreted as free acids, but in combination with glycine as hippuric acid (benzoyl glycine), $C_6H_5 . CO . NH . CH_2 . COOH$, and its homologue phenylaceturic acid, $C_6H_5 . CH_2 . CO . NH . CH_2 . COOH$.

The table shows that phenyl derivatives of fatty acids with odd numbers of carbon atoms have been oxidised to benzoic acid and those with an even number to phenylacetic acid. The oxidation of the fatty side chains, therefore, involves removal of *two* carbon atoms at a time, until the removal of two atoms is no longer possible, *i.e.*, we are left with either benzoic or phenylacetic acid. Both these acids are resistant to oxidation, for if administered they are excreted unchanged. Phenylvaleric acid would be oxidised first to phenylpropionic acid, which would then be oxidised to benzoic acid. This type of oxidation is called *β-oxidation*, since the β-carbon atom is oxidised to —COOH. Evidence supporting the theory of β-oxidation is not lacking.

(1) All fatty acids in the body have an even number of carbon atoms. If β-oxidation occurred, one stage in their oxidation would yield butyric acid. In cases when fat is metabolised without simultaneous oxidation of carbohydrate, derivatives of butyric acid—acetoacetic and β-hydroxybutyric acids—are excreted in urine. (Acetone, which is also excreted, is derived from decomposition of acetoacetic acid.) Note that these acids are β-oxidation products of butyric acid.

(2) β-Oxidation of fatty acids can be achieved *in vitro* by warming their ammonium salts to 37° C. with H_2O_2.

(3) By perfusion of the liver with blood to which fatty acids with even numbers of carbon atoms have been added, acetoacetic acid is formed. It is not formed if " odd carbon " acids are perfused.

(4) The presence of a double bond is no obstacle to β-oxidation. The acid $C_6H_5 . \overset{\gamma}{C}H = \overset{\beta}{C}H . \overset{\alpha}{C}H_2COOH$ (phenylisocrotonic) might be

expected to give γ-oxidation at the double bond. Actually it is treated just like phenylbutyric acid in the animal.

The occurrence of β-oxidation may be regarded as proven. But what is the mechanism by which the two carbon atoms are removed? After administration of phenylpropionic acid to dogs and cats, Dakin isolated the corresponding ketonic, hydroxy and unsaturated acids,

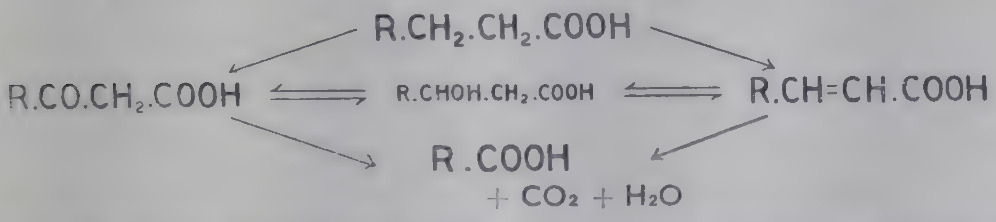
$$C_6H_5\,.\,\overset{x}{C}O\,.\,CH_2\,.\,COOH,$$
$$C_6H_5\,.\,\overset{x}{C}HOH\,.\,CH_2\,.\,COOH,$$
$$C_6H_5\,.\,\overset{x}{C}H = CH\,.\,COOH$$

(the β-carbons are marked x). The ketone $C_6H_5\,.\,CO\,.\,CH_3$ was also found, but is a decomposition product of $C_6H_5.CO.CH_2.COOH$. Note the analogy between these products and the derivatives of butyric acid excreted in urine in carbohydrate starvation referred to in (1) on p. 252.

$C_6H_5\,.\,CO\,.\,CH_2\,.\,COOH$	$CH_3\,.\,CO\,.\,CH_2\,.\,COOH$
$C_6H_5\,.\,CHOH\,.\,CH_2\,.\,COOH$	$CH_3\,.\,CHOH\,.\,CH_2COOH$
$C_6H_5\,.\,CO\,.\,CH_3$	$CH_3\,.\,CO\,.\,CH_3$

It is uncertain which is the primary oxidation product, for all three products are interconvertible. The final choice almost certainly lies between the ketonic and unsaturated acid, with a slight preference for the former. The results of the many experiments with different fatty acids are summarised below. The formation of hydroxy acid is represented in small type to indicate that it is probably a secondary reaction.

β-OXIDATION OF THE ACID $R\,.\,CH_2\,.\,CH_2\,.\,COOH$



In the original experiments the quantitative aspects of β-oxidation were not so satisfactory as were the qualitative. The yields of hippuric and phenylaceturic acids were of the order of 50%. Whilst a 100% yield could not be expected under the experimental conditions, one of only 50% suggested that β-oxidation could not alone be responsible for the oxidation of fats. We now know that aromatic acids are detoxicated by glucuronic acid as well as by glycine to such an extent as to account for some of the missing 50%.

The possibility of other types of oxidation, however, is not definitely excluded ; α-, γ- and δ-oxidation can, in fact, be demonstrated *in vitro*. There is some evidence to support Jowett and Quastel's view that oxidation can proceed *simultaneously* at alternate carbon atoms (β, δ, ζ, θ, κ, etc.) along the fatty acid chain. The oxidation product, which can be represented $CH_3.CO.CH_2.CO.CH_2.CO......CH_2.COOH$, would break up into molecules of acetoacetic and acetic acids. It has however, recently been shown that acetoacetic acid can be formed from acetic acid. In the case of the triglyceride of capric acid, $CH_3(CH_2)_8$ $COOH$, its administration has been shown to give rise to small amounts of sebacic acid, $COOH.(CH_2)_8.COOH$, in urine, suggesting the possibility of ω-oxidation.

Ketogenesis

Reference has been made to the appearance of acetoacetic acid, β-hydroxybutyric acid and acetone in urine in conditions in which there is a lack of carbohydrate, or inability to oxidise glucose (*e.g.*, starvation, diabetes mellitus). This suggests that the ordinary process of β-oxidation cannot be applied to butyric acid and that carbohydrate is in some way involved in the final oxidation of fatty acids. The formation of acetoacetic acid, β-hydroxybutyric acid and acetone (usually called the *acetone bodies*) in quantity * may be dangerous or even fatal, for apart from any intrinsic toxicity, two of the substances are fairly strong acids which have to be neutralised before excretion. The organism may be unable to supply alkali for this without seriously depleting the fixed alkali necessary for respiration. Some animals (*e.g.*, dogs and many carnivora) have rapid adaptation against this type of poisoning (*acidosis*), but others, man in particular, have not. It is consequently important that attention should be given to this aspect of fatty acid katabolism.

The formation of acetone bodies, **ketogenesis**, only occurs in conditions or diseases in which it can be shown that there is a lack of available carbohydrate, and will always be associated with **ketosis** (acetone bodies in blood) and **ketonuria** (excretion of acetone bodies in urine). The ketosis and ketonuria disappear when carbohydrate is given (provided it can be utilised, see p. 232). This effect of carbohydrate in checking ketogenesis is known as **antiketogenesis** and the carbohydrate as an antiketogenic substance. In starvation ketogenesis will only occur when all glycogen has been used up.

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* In severe diabetes mellitus more than 50 g. of acetone bodies may be excreted per day.

The exact mechanism of the reaction is not known. Since it can be shown *in vitro* that the oxidation of acetoacetate by H_2O_2 is greatly accelerated if glucose is added, it has been supposed that acetoacetic acid combines with some product of glucose metabolism to form a highly oxidisable compound, for there is evidence of a molecular relation between the two substances. By studying the acetone bodies produced during starvation and in diabetic subjects on special diets, it has been calculated that one molecule of glucose is able to complete the oxidation of two molecules of acetoacetic acid or two molecules of fatty acid (assuming one molecule of acetoacetic is derived from one molecule of fatty acid). Glucose may be replaced by other antiketogenic substances. The antiketogenic value of glycerol is half that of glucose, *i.e.*, one molecule of glycerol will serve one molecule of fatty acid. Therefore for fat, since one molecule of fatty acid is served by the glycerol, only one molecule of glucose is required to serve the other two fatty acids. Amino-acids may be ketogenic or antiketogenic, depending on whether they can give rise to glucose in the organism (a list is given on p. 272). If the ratio $R = \frac{\text{ketogenic molecules}}{\text{antiketogenic molecules}}$ of a diet is greater than 2, appreciable ketosis ensues. R is given by $R = \frac{2{\cdot}4P + 3{\cdot}43F}{3{\cdot}2P + 0{\cdot}57F + 5{\cdot}56G}$, P, F and G being the grams of protein, fat and glucose metabolised. These calculations have been applied with success in clinical practice; this offers some support for the molecular correctness of the assumptions made. Clinically, the rule that F must not exceed $2C + \frac{1}{2}P$ (F, C and P being the fat, carbohydrate and protein content of the diet) is more convenient than the formula for R in constructing diets.

It may be questioned whether there is a definite antiketogenic reaction. Ketogenesis occurs when the organism is forced to burn excess fat. But it may be reasonably argued that the animal has a limit to the proportion of fat it can metabolise; if this limit is exceeded, incomplete oxidation of fat would result. If the strain thus thrown on the fat metabolism be relieved by allowing other energy-producing mechanisms to function (*e.g.*, by giving carbohydrate), the fat will be oxidised completely again. Thus the action of antiketogenic substances may be due to their *fat-sparing action*.

The process may be compared to the reactions occurring in muscle. We only find lactic acid accumulating in blood and excreted in urine in severe exercise, but in this case we know that the reason for its appearance is that its production in the muscle is more rapid than its removal. If we relieve the strain on the muscle (*i.e.*, by rest) the lactic acid disappears. It is, therefore, not unreasonable to suppose that acetoacetic acid is a normal product of fat metabolism which is only revealed when the metabolic mechanism is strained. Such an explanation renders the possibility of adaptation to ketogenesis by training more understandable.

The Utilisation of Fat in Muscle

In man experiments involving determination of the respiratory quotient and blood fat of fat-fed or fasting individuals suggest that muscular exercise can be accomplished at the expense of fat. Whether the fat is utilised directly in the muscle or previously converted into carbohydrate is much disputed. Our present knowledge of the detailed processes of muscular contraction point to carbohydrate as the chief source of energy for muscular activity, but it must be remembered that not only is our knowledge in this field obviously incomplete, but also that the majority of *ex situ* experiments have been performed on muscles of small animals, frogs in particular. The experiments on respiratory quotient have been performed on the intact animals and man. The possibility of the conversion of fat to carbohydrate actually in the muscle has not been eliminated. For the moment the question must be left open.

Intermediary Metabolism of Cholesterol

Little is known of the fate of cholesterol which has been absorbed. Excessive feeding of cholesterol leads partly to its deposition, usually as esters, in many tissues and in liver with large amounts of triglycerides (p. 249). The fate of the remainder is unknown. A definite metabolic connection between cholesterol and the closely related bile salts, sex hormones, etc., has yet to be established. Another fact which awaits explanation is the occurrence of free cholesterol in varying amounts in different tissues, yet in approximately constant amount in individual tissues. There appears to be some definite phospholipide : cholesterol ratio for each tissue. The distribution of cholesterol in different types of muscles is roughly paralleled by their spontaneous activity. Cardiac and smooth muscles, which have a relatively extensive nervous mechanism, have a higher cholesterol content than voluntary muscles. (There is at present no way of distinguishing between the cholesterol in nervous tissue and that in the rest of the muscle.)

Cholesterol can be readily synthesised in the body. Experiments with heavy water suggest that cholesterol is built up from small molecules and not from other sterols.

CHAPTER XIX

UTILISATION OF PROTEINS

GENERAL (1, 4, 6, 8, 28, 36, 46)

In this country the average amount of protein consumed is about 80 g. per day (p. 388). This amount will include an appreciable quantity of conjugated protein (*e.g.*, hæmoglobin and nucleoprotein in meat), so that hydrolysis of our daily protein will provide not only amino-acids but compounds derived from the prosthetic groups, *e.g.*, phosphoric acid, sulphuric acid, purines, pyrimidines, hæmatin, etc. For the utilisation of the substances not discussed in this and the two succeeding chapters. see the index.

DIGESTION

Gastric Juice. There is no digestion of proteins in the mouth. The first digestion of protein takes place in the stomach under the influence of the gastric juice, which contains the enzymes pepsin and rennin.

Pepsin. It is usually stated that pepsin is secreted from the *Hauptzellen* of the gastric mucosa in its zymogen form—pepsinogen. Other cells, the parietal cells, secrete HCl, and when the two secretions mix the pepsinogen is converted into active pepsin.

$$\text{Pepsinogen} \xrightarrow{\text{Acid}} \text{Pepsin.}$$

Both pepsinogen and pepsin have been obtained in the form of crystalline proteins, and crystalline pepsin has been prepared by the action of HCl on crystalline pepsinogen. The optimum pH of pepsin is 1·2. This is attained by the HCl secreted, which amounts to about 0·4%. At a pH in the neighbourhood of that of normal gastric juice (about pH 1·4) pepsin rapidly attacks proteins, converting them to proteoses and peptones. While the food is in the stomach (two to four hours) a considerable amount of peptone is formed. The proportions of proteoses and peptones vary according to the conditions; usually there is more proteose than peptone. Pepsin is unable to hydrolyse proteins further than peptones. Of the scleroproteins, collagen is converted into gelatin, but elastin is attacked so slowly that it can be regarded as indigestible.

Rennin was the name originally given to the enzyme obtained from the fourth stomach of the calf, which had the property of causing milk to clot. It is familiar in the kitchen as the *rennet* for making junket from milk. The sole function of rennin seems to be the conversion of caseinogen of milk to casein, which is precipitated in the presence of calcium ions (see p. 413). Since the neutralised gastric juice of adults will clot milk, it has been argued that the juice contains pepsin and rennin, a view which can only be established by a complete separation of the two enzymes. So far, however, pepsin, even crystalline pepsin, has never been obtained devoid of rennetic activity; further, all proteases show rennetic activity, even the proteases of plants, which would never be called upon to deal with caseinogen. Highly purified rennin has no proteolytic activity and only converts caseinogen to casein. Milk could be clotted by pepsin as easily as by rennin. If the presence of rennin in adult gastric juice be insisted upon, it must at least be admitted that it is superfluous. Not so, however, in very young animals, where the pH of the stomach contents is much higher than it is in adults. A baby's gastric juice has a pH about 5, close to the optimum pH of rennin; it is actively rennetic but only weakly proteolytic. So far as digestion is concerned, milk is only clotted in the baby's stomach, the clot being passed on for further digestion to the small intestine. As the baby grows into a child the pH of the gastric juice decreases (becomes more acid) and its proteolytic activity increases, rendering rennin progressively less useful.

Pancreatic Juice. The acid mixture of proteoses and peptones and any undigested protein on leaving the stomach is neutralised by the pancreatic juice and bile in the duodenum, and is broken down into simple peptides and some amino-acids. (Some peptides, especially dipeptides, appear to be resistant to the action of pancreatic enzymes and are only split to amino-acids in the small intestine by a high concentration of erepsin.) There are three proteolytic enzymes in pancreatic juice, trypsin, chymotrypsin and carboxypolypeptidase. All three have been isolated as crystalline proteins. Trypsin and chymotrypsin are secreted in their inactive zymogen forms, trypsinogen and chymotrypsinogen, which have also been crystallised. Trypsinogen is activated by enterokinase * produced in the intestinal mucosa and chymotrypsinogen is activated by trypsin. Trypsin and chymotrypsin digest proteins to polypeptides which are further hydrolysed to simple peptides or amino-acids by carboxypolypeptidase. Both trypsin and chymotrypsin clot milk. The proponents of rennin claim that it is present in pancreatic juice.

Intestinal Juice. The chief enzyme of intestinal juice is erepsin, optimum pH 7·7. This acts on polypeptides and dipeptides, hydrolysing them to amino-acids. It is actually a mixture (which can be resolved) of aminopolypeptidase and dipeptidase. There are probably several proteases present in intestinal mucosa, and these are responsible for the alleged action of " erepsin " on proteins such as caseinogen. The intestinal juice and mucosa have so complex a mixture of enzymes that it is not surprising that the properties of the individual enzymes are not clear.

The mechanism for digestion of proteins is very efficient, even to the extent that the gastric and pancreatic enzymes can be withheld without serious consequences. Proteins are digested by gastrectomised individuals without difficulty, and also by pancreatectomised animals. In some animals it is possible to remove seven-eighths of the small intestine without seriously impairing the utilisation of protein. In all these cases, of course, more time is needed for digestion, and easily digestible protein must be selected for ingestion.

ABSORPTION AND TRANSPORT (39)

We now know that completely digested protein in the form of amino-acids is absorbed directly into the capillaries of the villi and passes *viâ* the portal vein to the liver. There is no evidence for absorption and transport of amino-acids by lymph. Being soluble and easily diffusible, the passage of amino-acids into the capillaries calls for no special explanation.

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* The *rôle* of enterokinase appears to be that of overcoming some inhibiting substance which is present in the pancreatic juice or crude trypsinogen preparation since crystalline trypsinogen in neutral solution forms trypsin spontaneously.

Our present knowledge of the absorption and transport of protein in the body was not attained without much labour and dispute, and can only be said to have been finally proved about twenty-five years ago. Some aspects of the early arguments are of interest, for the conception of the absorption and transport of proteins in the form of soluble protein or peptone was exceedingly difficult to disprove. This concept persisted owing to the difficulty of detecting any increase in the amino-acids of the blood after a protein meal, and was supported by the disappearance of proteins from isolated loops of intestine. But even after the discovery of proteolytic enzymes in the small intestine made it evident that protein was completely hydrolysed before absorption, it took ten years finally to refute the hypothesis that the amino-acids were resynthesised after absorption and transported round the body as soluble proteins. In 1912 Folin and van Slyke developed methods for estimating minute amounts of amino-acids (as amino-acid nitrogen). On applying these methods to blood, they proved that not only did the blood contain amino-acids, but also that the amount was increased after ingestion of protein. The actual amounts were very small. (Human blood contains from 3 to 8 mg. per 100 c.c. in the fasting state, rising only by 2 or 3 mg. after a meal.) A little later the amino-acids were actually isolated by Abel by the device of diverting an artery of an anæsthetised dog through a long collodion tube which was immersed in isotonic saline. In this way diffusible substances in the circulating blood could be abstracted by dialysis through the collodion wall of the tube into the surrounding saline. By employing this "*vividiffusion*" for 112 hours in all (*i.e.*, by adding the dialysates of a number of experiments), amino-acids corresponding to 1·5 g. of nitrogen ($\equiv$ 15 g. acids) were obtained and pure alanine and valine isolated from the mixture.

It may seem incredible that a rise of 3 mg. per 100 c.c. of blood is enough to account for the total amount of protein absorbed. A simple calculation will show that it is sufficient. Assuming a man takes 80 g. of protein a day, he will require $\frac{80,000}{3} \times 100$ c.c. $= 2,667$ litres of blood for its transport. As the volume of blood passing through the heart per day under resting conditions is about 8,640 litres (taking output of heart as 6 litres per minute), there is ample margin, for the figure of 3 mg. does not really indicate the true position, since amino-acids disappear from blood with great rapidity. Van Slyke found that when 12 g. of alanine were injected intravenously into a dog, 10·5 g. disappeared in five minutes.

The conditions of protein absorption are summarised in a recent experiment by Kúthy, who tested the rate of absorption of 7 g. of raw meat in rats. At each point in the curves (Fig. 21) two rats were killed and analysed for protein N left in the gut (to give the amount of meat absorbed) and amino N in the blood; the O_2 consumption was measured on other rats similarly fed in a closed circuit respiration apparatus. The curves show not only the parallel between absorption and the concentration of amino-acids in the blood, but also in the O_2 consumption, *i.e.*, the specific dynamic action of amino-acids (see p. 378); it is noteworthy that the experiment was performed under strictly physiological conditions on spontaneously fed animals.

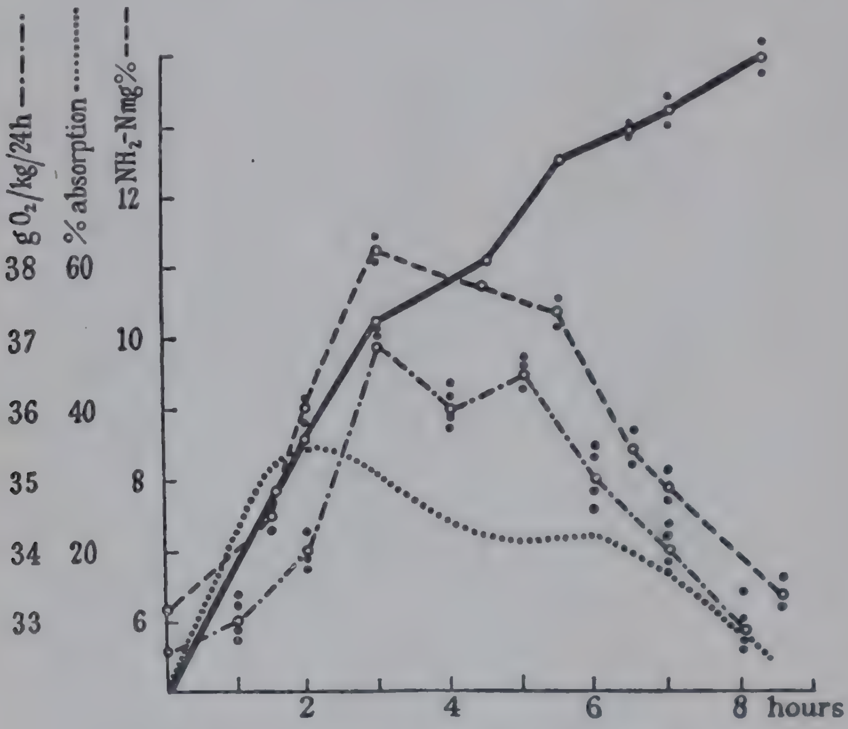


FIG. 21. Absorption of raw meat by rats.
——— Absorbed quantity.
.... Absorption rate per hour.
- - - - - - Amino nitrogen in blood.
- . - . - . Oxygen consumption.

(From Verzár and McDougall, "Absorption from the Small Intestine" (Longmans), 1936.)

The Possibility of the Absorption of Large Molecules

(*a*) **Proteins.** Whilst there is no doubt that practically all protein is absorbed as stated above, there is the possibility of absorption of very small amounts of soluble protein or partly digested protein under conditions of inhibited digestion. A case in point is that raw egg protein, which, it is stated, can be given without exciting gastric secretion and is resistant to the action of trypsin, can be shown to be slightly absorbed. The conditions under which such absorption occurs are, however, definitely abnormal. There is evidence of protein absorption under normal conditions in allergic individuals who are sensitive to ingestion of special proteins such as those of certain fish, or strawberries. Since normal men, sensitised with the serum of individuals allergic to a particular protein, showed allergic symptoms on eating that special protein, it must be concluded that minute amounts of protein may be absorbed. There is also evidence of slight protein absorption in new-born infants. Normally direct absorption of protein is so minute as to be quite unimportant in metabolism. The only effect might be that of the toxicity of a foreign protein.

(*b*) **Polypeptides.** London has recently produced experimental evidence that polypeptides are absorbed as well as amino-acids. The polypeptide N of blood in the portal vein rises as well as the amino-acid N during protein digestion. There is no evidence of synthesis of polypeptides from amino-acids after absorption.

INTERMEDIARY METABOLISM OF PROTEIN

The Disposal of the Absorbed Amino-acids

Light has been thrown upon the fate of absorbed amino-acids by studying the amino-acid content of various organs after administration of amino-acids or protein. *Magnified* results are obtained by injecting large amounts of amino-acids into an animal in which a nitrogen want has been created by twenty-four hours' *starvation.* Values in mg. per 100 g. obtained by van Slyke were :—

	Before Injection	About 1 Hour after Injection	3 Hours after
Liver . .	34	85	39
Muscle .	45	68	67

Tissues such as the muscles take up amino-acids to a certain point and retain them. The liver takes up amino-acids more greedily, but loses them again.

Now compare these results with those obtained after natural digestion of protein in a *normally fed* animal, *i.e.*, *unmagnified* results. Under these conditions there is no appreciable increase in the concentration of amino-acids in the tissues even in the liver, but there is a greater fall in concentration of amino-acids in blood passing through the liver than through any other tissues. This indicates that the liver can rapidly dispose of amino-acids. Since there is no appreciable increase in the liver amino-acids during natural digestion we can conclude that the amino-acids are utilised at the same rate as they are abstracted from the blood by the liver. The blood amino-acids, however, never fall to zero. Even in prolonged starvation there is practically the same amount as at the normal fasting level (3–8 mg. per 100 c.c. in man).

Parallel with the disappearance of amino-acids from the liver there is an increase in the percentage of urea in blood, followed by excretion of urea in the urine. The amino-acids abstracted by the liver are, in fact, rapidly converted into urea. (A small part may, however, be used for repair or special synthesis. See (2) and (3) on p. 265.)

The amino-acids abstracted by the tissues are used for their repair, for there is a constant breakdown and replacement of tissue proteins ("*wear and tear*"). Proteins characteristic of a given tissue are synthesised from the appropriate amino-acids, for the composition of a particular tissue protein often differs greatly from that of the food proteins. The unwanted amino-acids are presumably used up by other tissues or converted to urea in the liver as they pass round the circulation again. There is no storage of amino-acids in the tissues, for, even after injection of excessive amounts, amino-acids are not retained in the tissues (that is in normal, not *starved*, animals); the excess is excreted as urea.

There is yet another function of amino-acids. Since absorbed amino-acids form practically the sole source of nitrogen for the body, amino-acids must directly or indirectly provide the nitrogen for the synthesis of the many nitrogenous compounds of the body, such as the bile acids, creatine, purines, pyrimidines, hormones, amino-sugars, the bases of lipides, enzymes and the various bases found in tissues. Some of these, it is true, may be formed from urea rather than amino-acids, but the ultimate effect will be the same; amino-acids will be used without excretion of their nitrogen as urea.

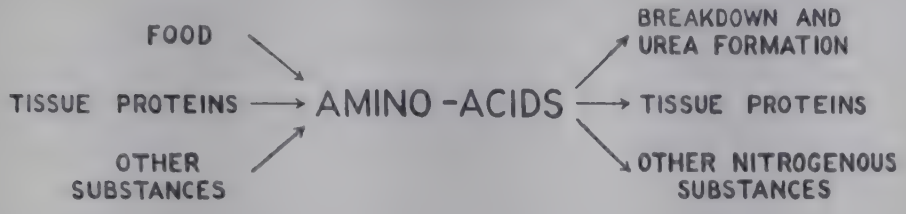
There are, then, three fates for amino-acids : (1) *conversion in the liver, so that urea is formed and then excreted* ; (2) *formation of tissue protein* ; (3) *formation of other nitrogenous substances.* (1) can be regarded as the fate of any amino-acids not used up in other ways, and the amount would vary with the protein supplied in the food. (2) and (3) are essential for the well-being of the body, but the substances formed cannot usually be stored. The extent of these reactions will therefore be independent of the protein supplied. Reaction (1), however, must not be thought of purely as a device for removal of excess unwanted amino-acids. It always goes on to some extent, even in starvation. If protein be given to a starved and emaciated animal, urea is excreted as soon as it would be in a well-fed animal. The starved animal does *not* first make good the depleted tissue proteins, and only excrete nitrogen (urea) when the tissues have been rebuilt to their normal condition.

It is customary, and beneficial, to eat considerably more protein than is really needed for the reactions (2) and (3). This ensures that there is a sufficiency of the right kinds of amino-acids for building tissue proteins. Any excess amino-acids cannot only be readily oxidised by the liver, but stimulate their own oxidation (see Specific Dynamic Action, p. 378). If the ingested protein only covers the needs of (2) and (3), tissue breakdown will be greater than repair, since reaction (1) always takes place to some extent. The essential and excess metabolisms of protein are usually differentiated by the terms **endogenous metabolism** and **exogenous metabolism.** Endogenous metabolism will cover reactions (2) and (3) and that part of (1) which proceeds independently of the food, as well as the normal breakdown (wear and tear) of tissue proteins. Exogenous metabolism is the metabolism of all protein ingested in excess of essential requirements. This convenient division of nitrogen metabolism is, however, really artificial since it is now clear, especially from isotope studies, that it is impossible to differentiate between the metabolism of amino-acids from tissues and those from ingested protein, even if the latter is far in excess of the body's requirement for reactions (2) and (3). Only small amounts of N^{15} are excreted after feeding amino-acids labelled with this isotope, more than half of this nitrogen being rapidly fixed in the tissue proteins.

It has been stated that no nitrogen is stored in the body. This requires further elaboration lest it should be thought that there can be no *retention of nitrogen.* In the healthy adult this does not ordinarily occur except on heavy protein diets; in other words, the nitrogen ingested is equal to that excreted in urine, fæces and sweat. Under these conditions the body is in *nitrogen equilibrium.* A healthy adult may retain nitrogen when he develops his muscles by doing muscular work over a period of several weeks and on an adequate diet. The extra tissue thus laid down is not *store* tissue, but extra *functional* tissue built up by training. In the same way there is retention of nitrogen in young animals during growth, for new tissues are being formed. When the diet is not sufficient to provide protein for the endogenous metabolisms, as in starvation, malnutrition or diseases in which food cannot be taken (*e.g.*, fevers), there will be a nitrogen loss which may involve considerable wasting of the muscles which have been used to provide energy in the absence of food. During recovery on an adequate diet the tissues are regenerated, and there will then be a nitrogen retention as in growth. The rapidity with which muscle tissue can waste and be regenerated is well illustrated in a child in fever and convalescence.

And now, for a moment, let us turn to the other important element of proteins—**sulphur.** There is a very close parallel between the metabolism of sulphur and that of nitrogen. There are three fates for the sulphur-containing amino-acids: (1) conversion in the liver, so that sulphuric acid and urea are excreted; (2) synthesis of tissue protein (including that of hair); (3) synthesis of other substances, *e.g.*, taurine, glutathione, insulin, thioneine and sulphatides. The endogenous metabolism of sulphur is represented by (2), (3), and to a certain extent (1), and the exogenous metabolism by (1). Similarly, under the conditions outlined above for nitrogen, there will be equilibrium, retention, or loss of sulphur. The excretions of urea and sulphuric acid (as sulphates) are closely parallel. Either can be regarded as reflecting exogenous protein metabolism.

To return to amino-acids in general, we have, passing into the circulation, amino-acids from the food, from breakdown of tissue proteins, and as by-products from other reactions; passing out of the circulation, we have amino-acids destroyed in the liver to produce urea, amino-acids synthesised into tissue proteins and amino-acids used in special syntheses :—



All these reactions presumably go on simultaneously, resulting in a balance between the opposing reactions, since the concentration of amino-acids in blood is very constant.

Of the synthesis and breakdown of tissue proteins we know very little except that they occur *in situ* under the influence of intracellular proteases.

The Breakdown of Amino-acids

The ultimate result of the breakdown of amino-acids is the separation of nitrogen, which is largely converted into urea, the residual carbon compounds being either oxidised to CO_2 and H_2O or used for synthesis of other substances such as glucose.

The first stage in the formation of urea is the separation of the amino-acid nitrogen as ammonia. This process is called **deamination** (or deaminisation). The ammonia formed is converted into urea.

I. Urea Formation

A. Site of Urea Formation. In the past, attention was focussed on the liver as the site of urea formation, chiefly for these three reasons :—

(1) Ammonium carbonate added to the blood perfusing an isolated liver is almost quantitatively converted into urea.

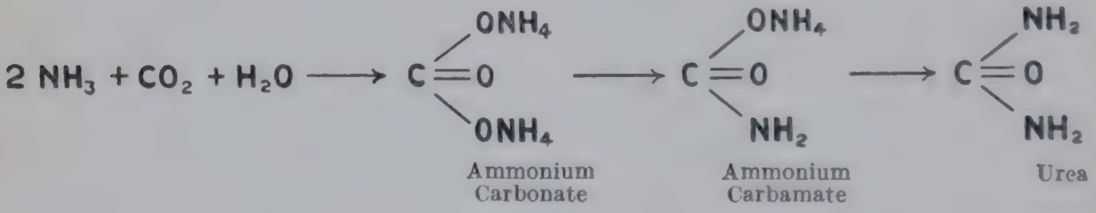
(2) In geese, in which uric acid is excreted instead of urea, extirpation of the liver results in diminution of uric acid excretion with a corresponding increase in elimination of ammonia.

(3) In mammals complete extirpation of the liver could not be achieved without killing the animal, and so recourse was had to diverting the portal blood to the inferior vena cava, and so partially removing the liver from the circulation (*Eck's fistula*).

Urea excretion diminished and ammonia increased, but urea formation never ceased, for the liver still functioned to some extent. Consequently there was doubt whether the urea came from the impaired liver or other organs.

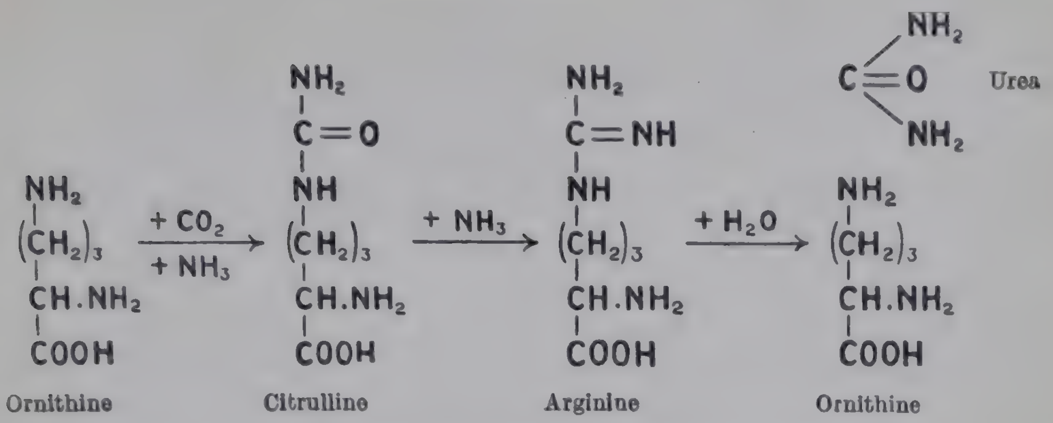
The question has been settled, for the dog, by Bollman and Mann, who were able to overcome the technical difficulties of liver extirpation sufficiently for the animals to survive the period of the experiment. They proved conclusively that in the dog urea formation occurred *only* in the liver. The final experiments involved removing both kidneys and liver and showing that the blood urea remained constant. If urea had been formed by other tissues the urea must have increased in the blood, since removal of the kidneys prevented its excretion. It is usually assumed that the results are applicable to man. They are, in fact, supported by an isolated clinical case of acute yellow atrophy reported by Rabinowitch. In this patient just before death, not only was the liver practically functionless, but the kidneys were so damaged as almost to prevent secretion of urine. The amount of urea in blood and urine was negligible, but the amino-acid content of blood was 216 mg. per 100 c.c. (normal value is 3–8 mg. per 100 c.c.), showing that deamination was also seriously affected.

B. The Chemical Mechanism of Urea Formation. The classical conception of the formation of urea from ammonia in the liver can be summarised in the following formulæ :—



$$2\,NH_3 + CO_2 + H_2O \longrightarrow C\begin{matrix}\diagup ONH_4\\ =O\\ \diagdown ONH_4\end{matrix} \longrightarrow C\begin{matrix}\diagup ONH_4\\ =O\\ \diagdown NH_2\end{matrix} \longrightarrow C\begin{matrix}\diagup NH_2\\ =O\\ \diagdown NH_2\end{matrix}$$

There are many objections to this simple explanation. Not the least is the absence from the liver of a suitable enzyme to effect this conversion. (The enzyme, urease, which reversibly catalyses the conversion of urea to ammonium carbonate, is very abundant in plants, but only occurs in small amount in the gastric mucosa of mammals.) The most satisfactory explanation so far involves the participation of ornithine in a continuous cycle. Ornithine combines with ammonia and CO_2 to form arginine, which breaks down to give ornithine and urea.



This scheme was proposed by Krebs on the basis of experiments studying the ammonia and CO_2 consumption, and urea production of slices of liver kept in a suitable oxygenated nutrient solution. Out of a very large number of amino-acids and nitrogenous bases tested, only ornithine and citrulline greatly accelerated the production of urea when added to the nutrient solution. From the quantitative behaviour of these two compounds it was deduced that citrulline was an intermediate product according to the scheme above. The conversion of arginine to ornithine and urea has long been known to be brought about by the enzyme *arginase.*

This attractive concept is supported by these main facts :—

(1) Out of seventeen tissues examined, liver was the only one to produce urea.

(2) Arginase is very abundant in mammalian livers, and is not found in any other organ except in the kidney in small amount.

(3) The scheme is supported by isotope experiments. It has been shown that the carbon of urea formed in tissue slice experiments can be derived from CO_2 by using radioactive CO_2 (C^{11}) or by using CO_2 labelled with C^{13}. Administration of N^{15} ammonia to living animals led to the excretion of isotopic urea and the arginine isolated from the proteins when acted upon by arginase gave isotopic urea but unlabelled ornithine.

(4) Arginase is not present in bird's livers, suggesting an explanation for their excretion of uric acid and not urea.

While this is undoubtedly the most satisfactory explanation of urea formation yet put forward, it must be remembered that the experiments upon which it is based are essentially *in vitro* experiments which await more physiological proof. It is possible that no single mechanism is responsible for the formation of urea. Recent experiments have, indeed, shown that urea can be formed in liver slices without the participation of arginase.

II. Deamination

The details of the chemistry of the apparently simple process of the removal of ammonia from amino-acids have yet to be discovered. The device employed by Knoop for studying the oxidation of fats—feeding phenyl derivatives of various amino-acids and noting the products excreted—has revealed that ketonic, hydroxy, and even fatty acids corresponding to the amino-acid may be produced. It is not known whether the ketonic or the hydroxy acid is the primary product. Since administration of dextrorotatory (+) phenyl aminoacetic acid led to excretion of lævorotatory (—) and not (+) phenyl hydroxy-acetic acid, Knoop argued that the hydroxy acid must have been a secondary product formed by asymmetric synthesis from some optically inactive intermediate, *e.g.*, by reduction of the ketonic acid thus :—

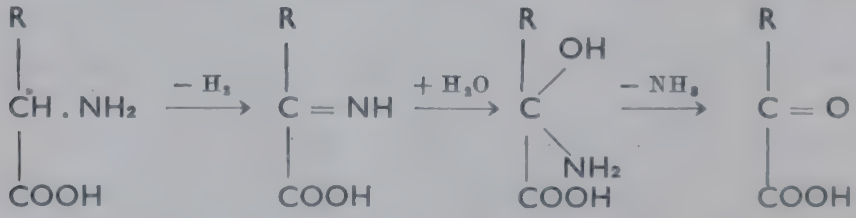
$$
d\text{-}C_6H_5\,.\,CH(NH_2)\,.\,COOH \rightarrow C_6H_5\,.\,CO\,.\,COOH \rightarrow \\
l\text{-}C_6H_5\,.\,CHOH\,.\,COOH
$$

and makes most improbable a deamination process :—

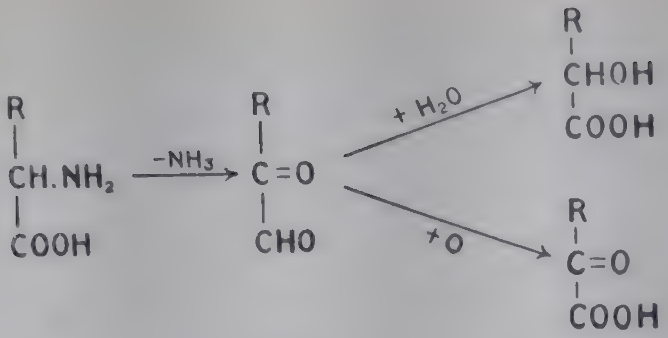
$$
R\,.\,CHNH_2\,.\,COOH + H_2O \rightarrow R\,.\,CHOH\,.\,COOH + NH_3
$$

It has since been shown, however, that (+) phenylaminoacetic acid has the same stereochemical configuration as (—) phenyl-hydroxy acetic acid, so that these acids are either both *d*- or both *l*- and not *d*- and *l*- respectively (see footnote on p. 62). The hydroxy acid could, therefore, be the primary product.

Knoop and Neubauer suggested that the deamination process should be represented :—

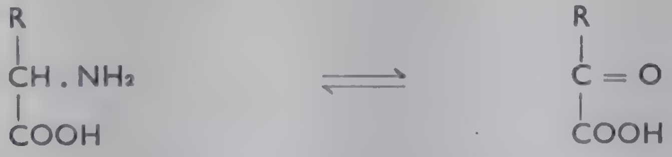


On the other hand, there is evidence to support Dakin and Dudley's contention that a ketonic aldehyde is the first stage. They showed that amino-acids in aqueous solution are always to some extent dissociated to the ketonic aldehyde and ammonia, and accordingly formulate deamination :—



Both representations fit the known facts. That of Dudley and Dakin has the additional merit that the liver is well supplied with an enzyme, *glyoxalase*, which converts α-ketonic aldehydes to α-hydroxy acids. Thus alanine would give in the first instance methyglyoxal, and thence pyruvic or lactic acid. (Note that these substances are important in sugar metabolism.)

Whatever the actual mechanism of deamination, it is certain that the amino-acids undergo α-oxidation with the elimination of NH_3. Before examining the fate of the carbon residue it must be made clear that the reverse reaction is possible. The synthesis of amino-acid from either the ketonic or hydroxy acid can be demonstrated. Administration of the α-ketonic acid of phenylbutyric acid not only gave the hydroxy acid, but also some amino-acid in the urine. Lactic or pyruvic acid added to blood perfusing an isolated liver yields alanine. Embden has obtained several different amino-acids in this way, so that the reaction can be assumed to be a general one and formulated (omitting the disputed intermediate stages) :—



$$\begin{array}{c} R \\ | \\ CH.NH_2 \\ | \\ COOH \end{array} \rightleftharpoons \begin{array}{c} R \\ | \\ C=O \\ | \\ COOH \end{array}$$

These reactions make possible the interconversion of carbohydrates and certain amino-acids.

III. The Fate of the Carbon Residue

The fate of the carbon residue depends on the nature of the amino-acid from which it was derived. Amino-acids can be divided into three classes according to the products formed when the acids are administered to animals rendered glycosuric by injection of phlorrhizin or by pancreatectomy. Such animals readily excrete glucose and acetoacetic acid, substances which would be completely oxidised in normal animals. After starvation to exhaust carbohydrate reserves, these animals will excrete glucose if certain amino-acids are administered, *i.e.*, glucose has been formed from these amino-acids, the process being described as **glucogenesis** or **gluconeogenesis.** Other amino-acids, however, give rise to acetoacetic acid ; yet others produce neither. The first group of amino-acids being glucogenetic will be *antiketogenic*, the second *ketogenic*. While these reactions can only be demonstrated in the glycosuric, it is believed that they can take place in the liver of the normal animal. The extent to which they take place will depend on the food absorbed. On an adequate diet with excess of protein a large proportion of the glucogenetic amino-acids would be oxidised directly to CO_2 and H_2O ; acetoacetic acid is probably a normal stage in the oxidation of the ketogenic amino-acids to CO_2 and H_2O. In a deficiency of carbohydrate the glucogenetic amino-acids would tend to form glucose. The behaviour of the different amino-acids is as follows:—

Antiketogenic	*Ketogenic*	*Neither*	*Unknown*
Glycine	Leucine	Valine	Threonine
Alanine	Isoleucine ?	Tryptophan	Norvaline
Serine	Phenylalanine	Lysine	Norleucine
Cysteine	Tyrosine	Histidine	Methionine
Aspartic Acid			
Glutamic Acid			
Hydroxyglutamic Acid			
Ornithine			
Arginine			
Proline			
Hydroxyproline			

The quantitative effect of the glucogenetic amino-acids can be calculated from the extra glucose and extra nitrogen excreted (the G/N ratio, p. 230). This provides further evidence that in the conversion of amino-acids to glucose three-carbon compounds are intermediates, for only 3 carbon atoms in each glucogenetic amino-acid are convertible into glucose. The glucogenetic value of proteins will, of course, depend on their content of glucogenetic amino-acids. It has been found experimentally that about 48% of caseinogen and 80% of gliadin is glucogenetic.

Some deaminated amino-acids may be reconverted to amino-acids. It has been shown *in vitro*, for example, that glutamic and aspartic acids can transfer their amino groups to ketonic acids in this way. Isotopic studies with N^{15} have shown that this process of **transamination** occurs readily in the body, particularly with certain amino-acids, *e.g.*, glutamic acid, aspartic acid, alanine, glycine, leucine, arginine, histidine, proline and tyrosine. When an amino-acid with N^{15} is fed to a rat much of the N^{15} is rapidly transferred to amino-acids of the tissue proteins. This process must involve the rapid breaking and reforming of the peptide linkages of the proteins. Lysine and ornithine do not undergo transamination. Glutamic acid and aspartic acid are especially active in accepting isotopic nitrogen. It has been estimated that in the rat about one half of all the amino-acids undergo transamination within three days.

In addition to their glucogenetic or ketogenic ability, some amino-acids may have special functions. These are described in Chapter XX.

The Production of Ammonia

While ammonia is liberated in the deamination of amino-acids, there is also a mechanism for its immediate conversion into urea. Now one of the most important defence mechanisms of the body is the production of ammonia for the neutralisation of acids and excretion of ammonium salts so as to spare the fixed base (Na, K, Ca) necessary for the alkali reserve and bones. It is typical of any *acidosis* (abnormal acidity of the blood) from whatever cause (*e.g.*, starvation, diabetes mellitus, acid poisoning) that there is an *increased excretion of ammonium salts* ; conversely, in alkalosis their excretion is decreased. Normally about 0·7 g. of ammonia is eliminated per day. It would be expected, therefore, that the blood would contain appreciable amounts of ammonia. Actually the amount is so small that its very existence has been disputed ; it is usually taken as 0·1 mg. per 100 c.c.

The kidney does not possess abnormal powers of concentrating ammonium salts, since extirpation of the kidneys does not lead to a rise in blood ammonia, so we must conclude that the kidney is responsible for the production of the ammonia of urine. This is supported by the fact that blood in the renal vein contains more ammonia than blood elsewhere. (In clinical nephritis a diminution of ammonia excretion is also observed.)

How does the kidney form ammonia ? There are two possibilities : (1) Ammonia is formed from urea. The strongest evidence for this is Bollman and Mann's experiments on hepatectomised dogs. In such animals no urea was produced. The urea and also the ammonia in the urine became extremely low, but on intravenous injection of urea, ammonia excretion was definitely increased. Amino-acids did not have this effect. Against this view is the difficulty of postulating any satisfactory mechanism, since the direct conversion of urea to ammonia must be ruled out owing to the absence of urease.

(2) Ammonia is formed from amino-acids. In contradiction to the experiments on hepatectomised dogs, perfusion of a dog's kidney with blood containing glycine led to an increase in ammonia content. Krebs has shown that kidney slices are even more active in deaminising amino-acids than are liver slices ; further, kidney slices could not convert urea into ammonia. This is supported by isotopic studies. When ammonia with N^{15} was fed to animals the greater part of the isotope was recovered from the urinary urea and only very little from the ammonia. When labelled urea was administered practically all the N^{15} was found in the urinary urea and practically none in the ammonia, but when labelled amino-acids were given the urinary ammonia had a much higher N^{15} content than the urea.

The possibility of the formation of ammonia from adenylic acid (p. 240) has also been suggested.

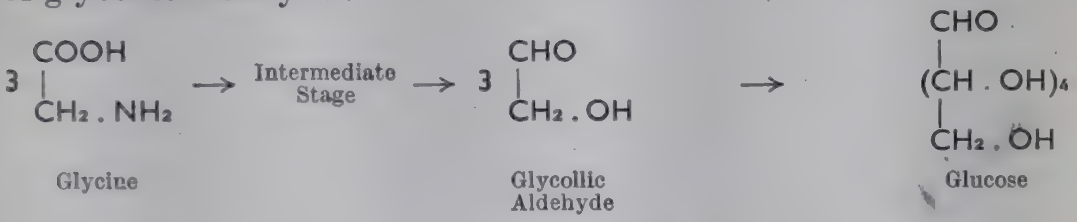
CHAPTER XX

UTILISATION OF PROTEINS (*contd.*)

METABOLISM OF INDIVIDUAL AMINO-ACIDS
(1, 4, 28, 36)

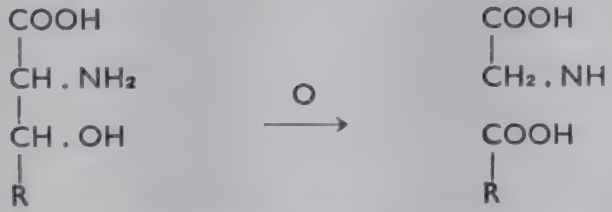
This chapter contains a lot of miscellaneous information, and would be better omitted until the reader has acquired a good general knowledge of the subject. It reveals how little we really know of amino-acid metabolism.

Glycine is somewhat atypical in having only two carbon atoms, yet all its carbon atoms are converted to sugar. The mechanism is still obscure. Possibly glucose is formed from three molecules of glycollic aldehyde :—



$$3\begin{array}{l}COOH\\ |\\ CH_2.NH_2\end{array} \longrightarrow \text{Intermediate Stage} \longrightarrow 3\begin{array}{l}CHO\\ |\\ CH_2.OH\end{array} \longrightarrow \begin{array}{l}CHO\\ |\\ (CH.OH)_4\\ |\\ CH_2.OH\end{array}$$

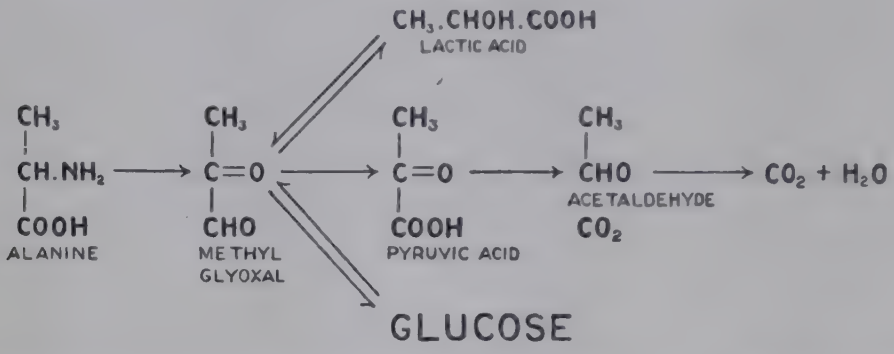
Glycollic aldehyde (and acid) yield glucose in the glycosuric animal. Other functions of glycine are its conjugation with cholic acid to form **glycocholic acid**, and its detoxication of aromatic acids such as benzoic acid (p. 315), with which it forms benzoyl glycine—**hippuric acid.** The animal can apparently synthesise glycine for this purpose in quantity. It may possibly be synthesised by β-oxidation of α-amino-β-hydroxy acids.



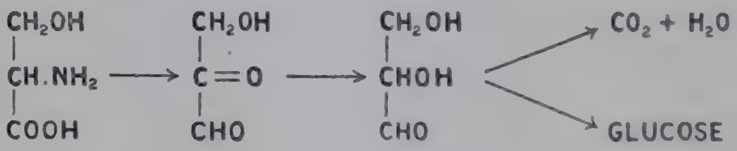
$$\begin{array}{l}COOH\\ |\\ CH.NH_2\\ |\\ CH.OH\\ |\\ R\end{array} \xrightarrow{O} \begin{array}{l}COOH\\ |\\ CH_2.NH\\ \\ COOH\\ |\\ R\end{array}$$

There is some evidence that glycine may be involved in the synthesis of creatine (p. 286). Glycine has a high specific dynamic action.

Alanine can be regarded as the typical amino-acid which has been considered in the general section. It yields glucose in the glycosuric animal, all the carbon atoms being converted. Methyl glyoxal is readily converted completely to glucose in the glycosuric animal, pyruvic acid not so easily. The metabolism of alanine may be represented :—


CH₃.CHOH.COOH
LACTIC ACID
CH₃ CH.NH₂ COOH
ALANINE
CH₃ C=O CHO
METHYL GLYOXAL
CH₃ C=O COOH
PYRUVIC ACID
CH₃ CHO
ACETALDEHYDE
CO₂
CO₂ + H₂O
GLUCOSE

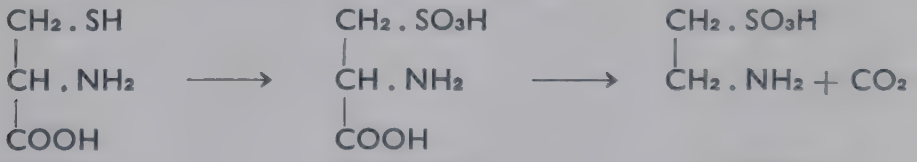
Serine is also quantitatively glucogenetic. Its metabolism is probably


CH₂OH CH.NH₂ COOH
CH₂OH C=O CHO
CH₂OH CHOH CHO
CO₂ + H₂O
GLUCOSE

It might also form glycine (*q.v.*).

Cystine is quantitatively glucogenetic, probably being reduced to cysteine and converted to serine. In its normal exogeneous metabolism the sulphur is oxidised to sulphate. Part of this sulphate is used to detoxicate phenols (ethereal sulphate, p. 316). Since cysteine (or cystine) and methionine form almost our sole source of sulphur, they must be regarded as precursors of the sulphur compounds of the body.

The formation of taurine may be represented :—



$$\begin{array}{l}CH_2.SH\\ |\\ CH.NH_2\\ |\\ COOH\end{array} \longrightarrow \begin{array}{l}CH_2.SO_3H\\ |\\ CH.NH_2\\ |\\ COOH\end{array} \longrightarrow \begin{array}{l}CH_2.SO_3H\\ |\\ CH_2.NH_2 + CO_2\\ \\ \end{array}$$

Cysteine Cysteic Acid Taurine

Glutathione and the proteins would be built up from amino-acids in the ordinary way. In the formation of hair there is evidence that growth of hair is diminished on low sulphur diets, and to a greater extent than the general growth of the animal.

Cystine cannot be replaced in the diet by acids in which the

NH_2 has been substituted by H, di-(β-thiolpropionic acid) or OH, di-(α-hydroxy-β-thiolpropionic acid), nor by taurine, cysteic acid, nor even by *d*-cystine (the natural form is *l*-cystine). It can, however, be replaced by methionine and its derivative homocysteine.

Cystine is used for detoxication of toxic substances such as bromobenzene, which is excreted as mercapturic acid (p. 318).

There is some endogenous metabolism of sulphur resulting in the excretion of "*neutral sulphur*," but its nature is unknown.

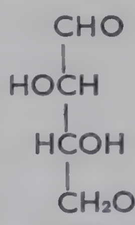
Cystinuria (49) is an abnormal condition in which cystine is always present in urine, often crystallising out, and is regarded as an inborn error of metabolism which is definitely hereditary. Cystine continues to be excreted even in starvation. Free cystine ingested is, however, katabolised normally without increasing the cystinuria. There is some evidence that the cystine of cystinuria is not free cystine, but a labile precursor which decomposes to give cystine as the urine stands. The cause of the abnormality is unknown.

For the activity of sulphydryl compounds in oxidation and reduction see p. 143.

Threonine. Ingestion of the phenyl derivative of this acid leads to the excretion of phenylacetic acid, whereas γ-phenyl-α-aminobutyric acid gives benzoic acid. If threonine acts in the same way, a possible source of glycine is suggested.

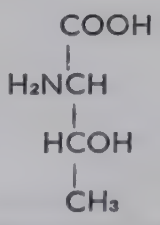
$$CH_3 . CHOH . CH(NH_2) . COOH \rightarrow$$
$$CH_3 . COOH + CH_2(NH_2) . COOH$$

The configuration of the natural form corresponds to *d*(−)threose (hence its name).



$$\begin{array}{c} CHO \\ | \\ HOCH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

d(−)THREOSE



$$\begin{array}{c} COOH \\ | \\ H_2NCH \\ | \\ HCOH \\ | \\ CH_3 \end{array}$$

THREONINE

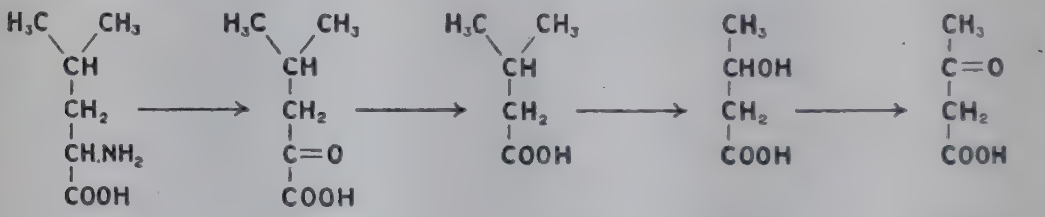
Threonine is an essential amino-acid and cannot be replaced by its optical isomers.

Methionine is an essential amino-acid which is convertible into cysteine. After administration of methionine containing radioactive sulphur, cystine with radioactive sulphur can be isolated from the tissue proteins. Note that replacement of the methyl group of methionine by hydrogen would give the next higher homologue of cysteine, *homocysteine*, $HS . CH_2 . CH_2 . CH(NH_2) . COOH$. This is possibly the first stage in its katabolism.

There is evidence of a metabolic relationship between methionine and choline. An animal cannot utilise homocystine as a substitute for methionine unless choline is administered. If methionine with its methyl group "labelled" with deuterium is fed to rats, choline with a deutero-methyl group can be isolated from the tissues. This suggests a reversible transfer (*transmethylation*) of methyl groups between methionine and choline. (Ref. 74.) The transfer of a "labelled" methyl group from methionine to guanidine acetic acid to form creatine has also been demonstrated (p. 286).

Valine. This essential amino-acid forms neither glucose nor acetoacetic acid, otherwise its katabolism is unknown.

Leucine. The production of acetoacetic acid from leucine may be represented :—

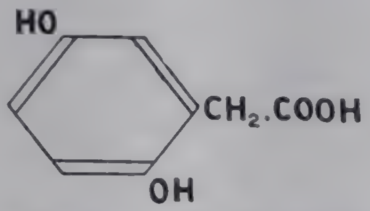


Leucine — Isovaleryl-formic Acid — Isovaleric Acid — β-Hydroxy-butyric Acid — Acetoacetic Acid

The acetoacetic acid would normally be oxidised in the usual way. Both leucine and isoleucine are essential amino-acids.

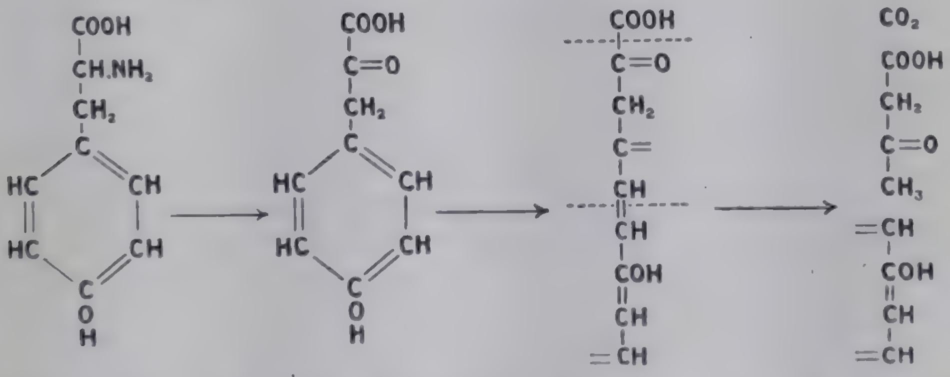
Phenylalanine and Tyrosine. These two amino-acids are probably closely related metabolically. If deuterophenylalanine is fed to rats, deuterotyrosine can be recovered subsequently from the tissue proteins. The reaction is apparently not reversible since phenylalanine is an essential amino-acid. Little is known of their complete katabolic degradation beyond the fact that phenyl- or *p*-hydroxyphenyl-pyruvic acid can form acetoacetic acid in the glycosuric animal. This means that the ring must open to provide the necessary carbon atoms. Two metabolic errors of the metabolism of these acids are known and throw some light on the early stages.

Alkaptonuria (49). This rare condition is characterised by the excretion of homogentisic acid which causes the urine to turn black on exposure to air.



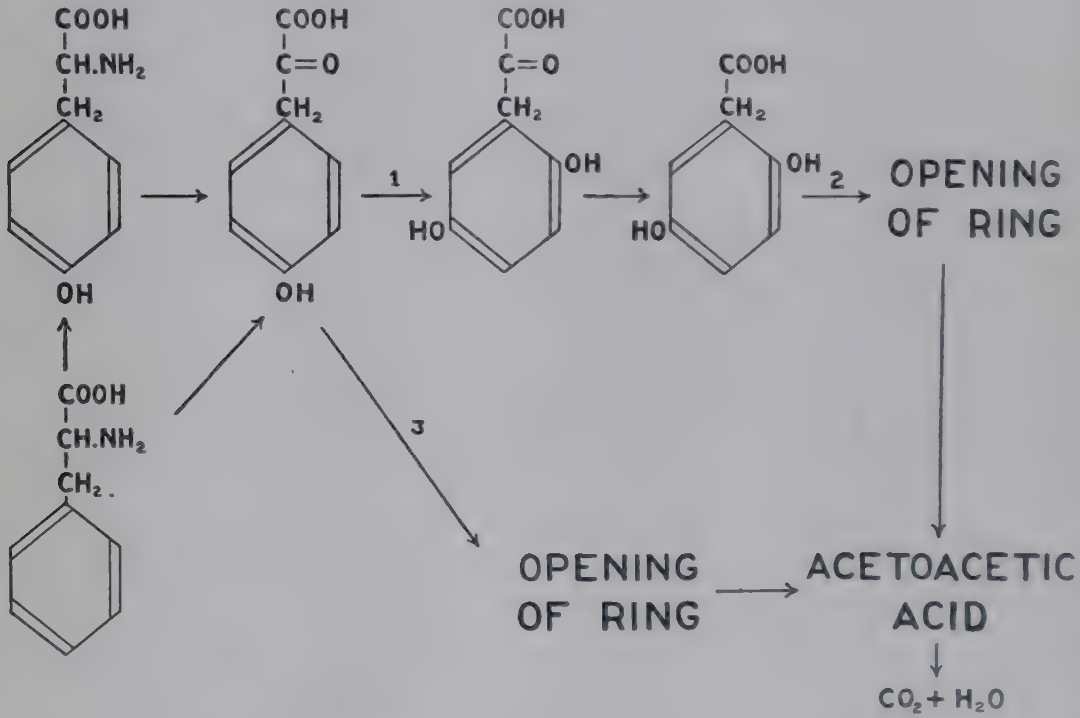
HOMOGENTISIC ACID

(The blackening on exposure to air is a reaction similar to that observed when pyrogallol is made alkaline in the presence of oxygen. The formation of these black pigments (melanins) is characteristic of many di- and tri-hydric phenols such as hydroquinone, which is excreted after absorption of phenols.) The condition of alkaptonuria is hereditary and more frequent in males than in females. There are apparently no associated ill-effects. Phenylalanine and tyrosine given to an alkaptonuric are excreted as homogentisic acid. Administered homogentisic acid is excreted unchanged. All three substances are completely katabolised in the normal individual, and form acetoacetic acid when perfused through a surviving liver. It is usually concluded that homogentisic acid is a normal stage in metabolism of phenylalanine and tyrosine, and that alkaptonurics are unable to metabolise this intermediate. Dakin, however, maintains that normally ring opening occurs and homogentisic acid is an abnormal product.



Tyrosinosis. In this condition, only one case of which is known, the tyrosine metabolism is checked at an earlier stage than in alkaptonuria, *p*-hydroxyphenylpyruvic acid being excreted.

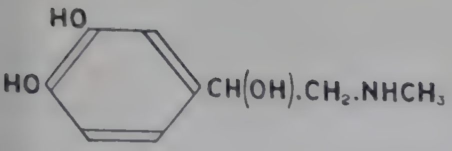
Feeding with phenylalanine or tyrosine increased the amount; some tyrosine was also eliminated even when phenylalanine alone was fed, thus stressing the close relationship between phenylalanine and tyrosine. Homogentisic acid was katabolised normally. This condition therefore suggests a failure of the mechanism for 2 : 5 oxidation of tyrosine and phenylalanine (or ring opening, according to Dakin's view). The facts from these two conditions could be represented :—



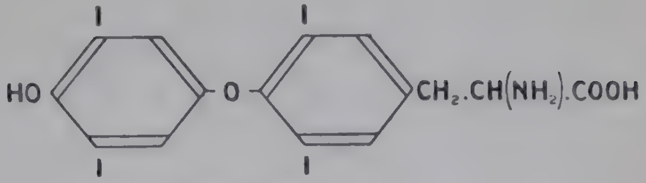
Step (1) or (3) (according to Dakin's view) cannot be performed in "tyrosinosis" and step (2) is impossible to alkaptonurics; (3) is Dakin's conception of the normal path of katabolism.

Phenylketonuria. Certain mental defectives excrete phenylpyruvic acid in urine. This condition, which is known as phenylketonuria or *imbecillitas phenylpyruvica* is inherited. The excretion of phenylpyruvic acid is increased when phenylalanine, but not tyrosine, is ingested This suggests that these individuals cannot oxidise phenylpyruvic acid at the normal rate and that phenylalanine has a metabolic path other than that *viâ* tyrosine.

In addition to their being components of proteins, tyrosine and phenylalanine can probably be regarded as precursors of the hormones adrenaline and thyroxine.


HO
HO
CH(OH).CH₂.NHCH₃

ADRENALINE


I
I
HO
O
CH₂.CH(NH₂).COOH
I
I

THYROXINE

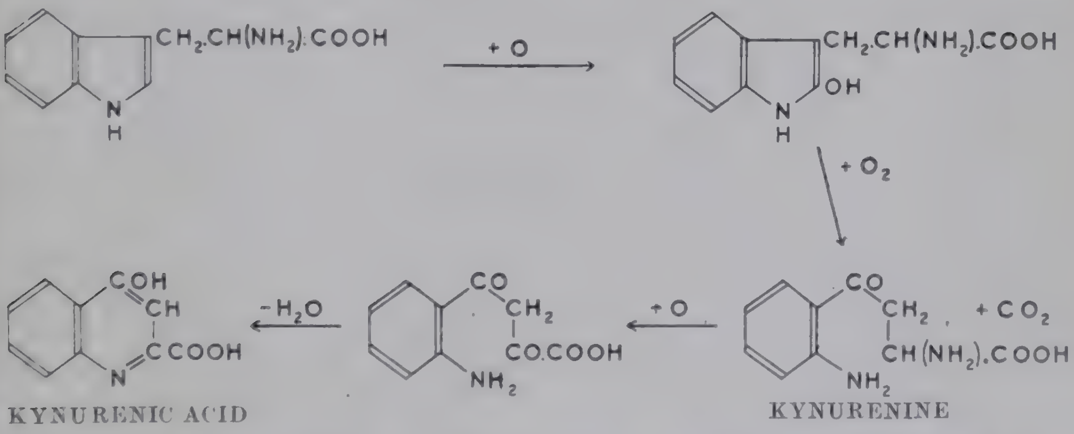
In vitro experiments (tissue slice) suggest that the guinea-pig is provided with enzymes for the changes

$$\text{TYROSINE} \xrightarrow[\text{Kidney}]{} \text{TYRAMINE} \xrightarrow[\text{Adrenals}]{} \text{ADRENALINE}$$

The black pigment melanin found in the retina, hair and sunburnt skin of white races and dark skin of coloured races is probably produced by oxidation of tyrosine by the enzyme tyrosinase. The oxidation of tyrosine has been worked out in detail by Raper, who showed that the oxidation involves the formation of dihydroxyindole and its carboxylic acid, which polymerises spontaneously to melanin.

For the bacterial putrefaction of phenylalanine and tyrosine to phenols and their detoxication see pp. **201**, **303**, **316**.

Tryptophan. Excess of tryptophan given to animals on a normal diet causes the excretion of kynurenic acid. In the absence of pyridoxin from the diet kynurenine is excreted in the place of kynurenic acid. The probable origin of these compounds is indicated in the following scheme.


CH₂.CH(NH₂).COOH
N
H
+ O
CH₂.CH(NH₂).COOH
OH
N
H
+ O₂
COH
CH
C COOH
N
- H₂O
CO
CH₂
CO.COOH
NH₂
+ O
CO
CH₂
CH(NH₂).COOH
NH₂
+ CO₂
KYNURENIC ACID
KYNURENINE

Complete breakdown of tryptophan to kynurenic acid is only possible in the presence of pyridoxin. It is likely that pyridoxin is included in the prosthetic group of an enzyme required for further oxidation of kynurenine. Tryptophan is an essential amino-acid.

For bacterial putrefaction and detoxication of tryptophan see pp. 201, 203, 316.

Aspartic Acid is glucogenetic to the extent of three carbon atoms out of the four, so that presumably some three-carbon compound is formed in its katabolism. The nature of this compound is disputed. Derivatives of succinic, fumaric and malic acids have been obtained under varying conditions. One of these presumably loses CO_2 to give a three-carbon compound, which is either further oxidised to CO_2 and H_2O, or built up into glucose.

Glutamic Acid is glucogenetic to the extent of three carbon atoms out of the five. Details of its katabolism are unknown. Glutamic acid is a component of glutathione. It can probably form proline (*q.v.*). It is particularly active in transamination (p. 273) and is the most abundant amino-acid in animal proteins.

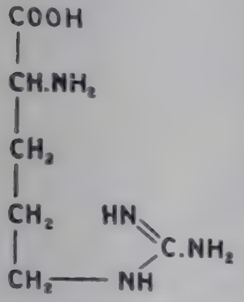
Hydroxyglutamic Acid probably behaves more or less like glutamic acid, and, being an α-amino-β-hydroxy acid, may be a source of glycine (*q.v.*). It is glucogenetic.

Arginine is glucogenetic to the extent of three carbon atoms out of the six. The first stage in its katabolism is ornithine and urea (p. 269). Ornithine is then supposed to form succinic acid and CO_2, and thence a three-carbon compound. The suggestion that arginine by oxidation and methylation would give creatine cannot be supported by any evidence. Its *rôle* in creatine formation is rather that of the donor of an amidine group to glycine (p. 286). For the possibility of purine formation from arginine see Histidine.

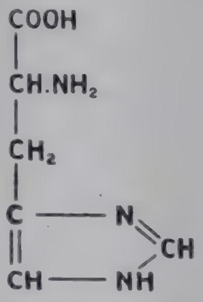
Arginine can be synthesised in the body (of the rat), but not fast enough for normal requirements. Hence it is regarded as an essential amino-acid.

Lysine is neither glucogenetic nor ketogenic. It is an essential amino-acid. Details of its metabolism are not known. It does not participate in transamination like other amino-acids (p. 273).

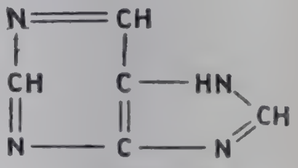
Histidine. It is doubtful whether histidine gives rise to more than very small amounts of glucose or acetoacetic acid. It is an essential amino-acid. The details of its katabolism are little known. Ingested histidine is almost completely utilised, only very small amounts of iminazoles being excreted. Iminazole acrylic acid (*urocanic acid*) has been found in dogs' urine after large doses of histidine. An interesting relation between arginine, histidine and purine was revealed by experiments of Ackroyd and Hopkins on rats. Removal of both bases from the diet led to diminished allantoin excretion (allantoin is the main end product of purine katabolism in the rat). This was prevented by addition of either base singly. This suggests that arginine and histidine may have a common katabolic path which may give rise to purine. Other workers, however, have produced evidence that histidine, but not arginine, can function as a precursor of purine. The relationship between the two bases and purine can be represented :—



ARGININE



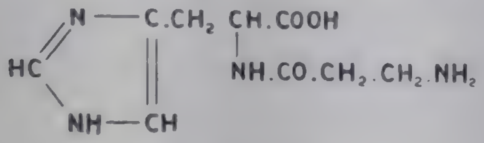
HISTIDINE



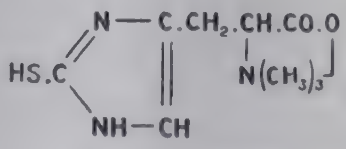
PURINE

Abnormally large amounts of histidine have been reported in the urine of pregnant women.

Two substances found in the body would be formed from histidine. **Carnosine**, a constituent of muscle, is a dipeptide, β-alanyl histidine. (Note it is β-alanine, *i.e.*, NH_2 group in β-position and not the α-alanine of proteins) ; **thioneine** or **ergothioneine**, found in red blood corpuscles, is the betaine of thiolhistidine. No function has yet been ascribed to either of these bases.

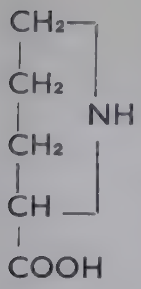


CARNOSINE

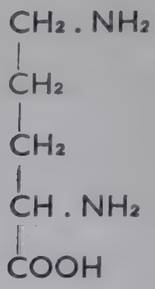


THIONEINE

Proline and Hydroxyproline are glucogenetic to the extent of three out of five carbon atoms. The stages in the breakdown are not definitely known, but presumably resemble those of ornithine or glutamic acid katabolism.

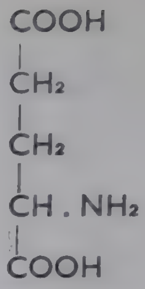


Proline



$$CH_2.NH_2-CH_2-CH_2-CH.NH_2-COOH$$

Ornithine



$$COOH-CH_2-CH_2-CH.NH_2-COOH$$

Glutamic Acid

It is usually assumed that synthesis of proline in the body is by way of glutamic acid, although it can apparently be formed from other sources, since the prolines, glutamic acid, arginine (ornithine) and aspartic acid can be simultaneously removed from the diet without ill-effects.

CHAPTER XXI

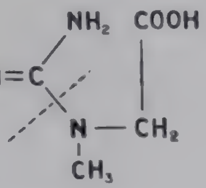
UTILISATION OF PROTEINS (*contd.*)

CREATINE AND NUCLEOPROTEIN

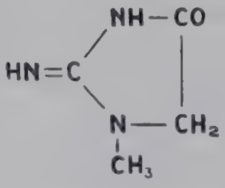
A. METABOLISM OF CREATINE AND CREATININE (1, 8, 47)

CREATINE, methyl guanidine acetic acid, is widely distributed in animal tissues. Skeletal muscle, in which it is most abundant, contains about 0·5% creatine, and heart muscle about half that amount ; 98% of the total creatine in the body is in the muscles. Its anhydride, creatinine, is excreted by the adult to the extent of about 1–1·5 g. per day. As the amount is not appreciably affected by the diet, creatinine is a product of endogenous metabolism.

The two compounds are readily interconverted in solution, as might be expected from their formulæ :—



CREATINE

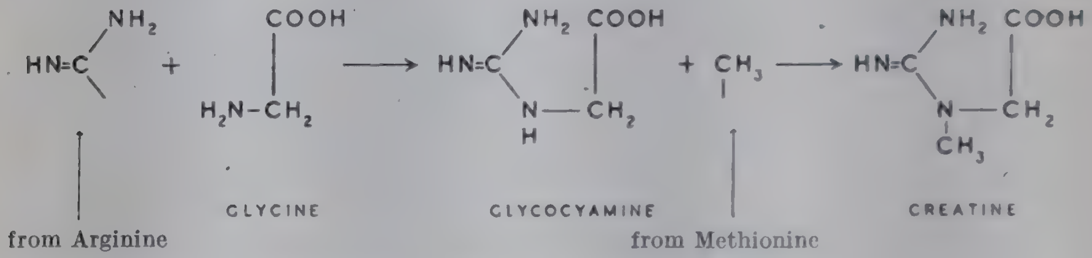


CREATININE

Acid favours the formation of creatinine, alkali of creatine.

The Origin of Creatine and Creatinine. Creatine is essentially a constituent of animal tissues, so that vegetarians and herbivorous animals will not ingest any appreciable amount. Creatine, in fact, can be completely eliminated from the diet without any ill-effects. It must, therefore, be formed from some product already in the body.

The guanidine group suggests a possible origin in arginine, but many attempts failed to reveal any metabolic connection between the two substances. Clinical observations, however, led Brand to suggest that glycine is a precursor of creatine. Note that the dotted line in the formula separates the skeletons of urea and N-methyl glycine (*sarcosine*). Patients with progressive muscular dystrophies excrete almost quantitatively any ingested creatine, so that it might be supposed they would excrete any precursor of creatine in the same way. On this basis Brand added to the diets of these individuals those nitrogenous substances which might conceivably give rise to creatine. Creatine excretion was only markedly increased when glycine or the glycine-rich protein, gelatin, was added to the diet ; if benzoic acid was also given the creatinuria was diminished. (Benzoic acid must be conjugated with glycine before it can be eliminated.) Glycine could be converted to creatine by receiving an amidine group from arginine and a methyl group from methionine or choline thus :—



This scheme is supported by the following facts :—

(1) Administration of guanidine acetic acid (glycocyamine) increases creatine excretion like glycine or gelatin.

(2) Glycocyamine is a normal constituent of tissues and urine.

(3) Tissue slice experiments have shown that the kidney can synthesise glycocyamine from glycine and arginine and that liver can rapidly methylate glycocyamine when methionine is added.

(4) Administration of N^{15} labelled glycocyamine to animals gave isotopic creatine and creatinine.

(5) N^{15} labelled glycine and arginine were the only labelled amino-acids which formed labelled creatine and creatinine. In these experiments the creatine obtained by glycine feeding was labelled on the sarcosine nitrogen only, whereas the creatine obtained by feeding arginine, which was labelled *only* on the two amidine nitrogens, was labelled only on the amidine group.

(6) The *rôle* of methionine and choline in the methylation was shown by administering these compounds with their methyl group hydrogens substituted with deuterium. When the methionine was given to rats deuterium was found in choline and creatine, and when the choline was fed deuterium was found in methionine and creatine.

The Fate of Creatine and Creatinine. The relationship of creatine to creatinine is almost equally obscure. It is barely ten years since the function of the large amount of creatine in muscle was discovered (p. 238). It is by now fairly generally conceded that creatine, but not creatinine, is present in the tissues and that creatinine is an end product of the metabolism of creatine, but not in the sense that urea is an end product of amino-acid metabolism, for creatinine is only excreted to the extent of about 2% of the creatine after the ingestion of large amounts of creatine, which is mainly excreted unchanged. Ingested creatine is not excreted quantitatively; part is always retained; small amounts are wholly retained. (There is no increase in urea or ammonia excretion.) Prolonged feeding with moderate amounts of creatine only gives rise to creatinine after a lag of several days. Ingested creatinine is rapidly excreted nearly quantitatively. The tissues of nephrectomised rats, after injection of creatine, retain large amounts in the liver and muscles, but some disappears. Isotope experiments support the view that creatinine is formed from creatine since after feeding labelled creatine the concentration of N^{15} in the creatine of the tissues and the creatinine of the urine is the same. Once formed, creatine does not transfer its nitrogen. Ingested isotopic creatine remains in the tissues unchanged or is excreted as creatinine. Creatinine is an end product which cannot be metabolised.

Undoubtedly the chief function of creatine is the formation of phosphagen, the phosphate reservoir for muscular contraction (p. 238). The connection between creatine and muscular metabolism is reflected when the muscles atrophy in starvation, fevers or the myopathies. In these conditions creatine is excreted by male adults who normally only excrete creatinine. Creatine is liberated whenever there is unusual breakdown of muscles, and presumably cannot be readily utilised as fuel along with the amino-acids of the tissue proteins; nor is it converted into creatinine.

The excretion of creatinine indicates that it is in some way a product of muscle metabolism. The daily excretion of creatinine is so constant for a given individual that its estimation is often carried out to ensure that complete twenty-four-hour samples of a patient's urine are being collected. The amount excreted is not, however, proportional to the *size* of the subject, but to his *muscular development*, a man with well-developed muscles excreting more than a less muscular man of the same weight. But ordinary *muscular exertion* does *not* affect the *daily* output of creatinine, which is approximately the same on a day of exercise as on a day of rest. (The daily excretion rises gradually over a long period when muscle tissue is increased by training.) The correlation between creatinine excretion and muscular exercise can, however, be shown by studying the *hourly* excretion. Then it is seen that exercise leads to increased excretion, which is, however, correspondingly diminished in the subsequent rest period, so that the ultimate effect is that of a constant daily excretion regardless of exercise.

The foregoing applies to a normal male adult. In the female there is intermittent creatinuria (excretion of creatine), which is continuous in pregnancy. Children of both sexes excrete both creatine and creatinine normally until puberty. This creatinuria cannot be satisfactorily explained as due to deficient muscular development, since creatinuria occurs with women who are highly trained physically. That there is probably some relation to sex is supported by the observation that creatinuria occurs in eunuchs, and may be induced in aged men by administration of *small* amounts of creatine.

B. THE METABOLISM OF NUCLEOPROTEINS, PYRIMIDINES AND PURINES (1, 8, 32, 33)

Small amounts of nucleic acids (as nucleoprotein) and purines are common constituents of foodstuffs, especially meats. Liver contains about 0·5% and muscle 0·2% of nucleic acid ; appreciable amounts are present in peas and beans ; dairy products, including eggs, are practically purine-free ; free purines are taken in the form of coffee and tea (caffeine) and cocoa.

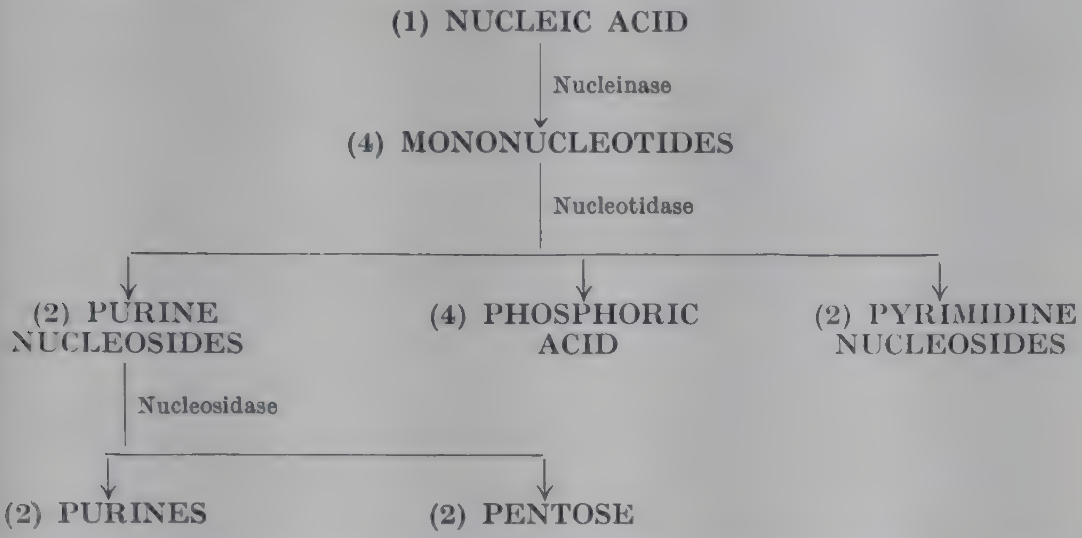
Digestion. Protein is successively removed from nucleoprotein by the gastric and pancreatic juices (p. 110). No further digestion of nucleic acid occurs until it reaches the intestinal juice which contains a series of special enzymes for its digestion. These are :—

Nucleinase (or polynucleotidase), splitting nucleic acid to mononucleotides.

Nucleotidase (or nucleophosphatase), splitting mononucleotides to nucleosides and phosphoric acid.

Nucleosidase, splitting purine nucleosides to purine and sugar.

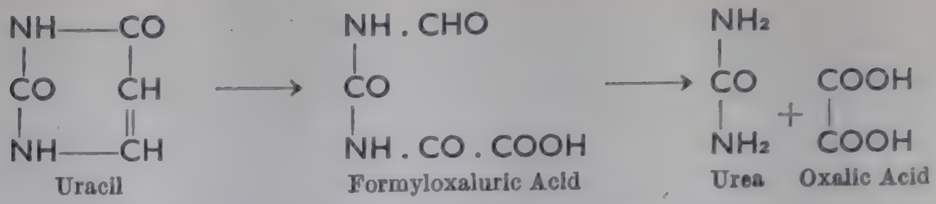
Pyrimidine nucleosides are apparently not hydrolysed further and are absorbed as such. All the enzymes are specific for the reactions indicated except nucleotidase which behaves as a typical phosphatase and splits glycero- and hexose-phosphates. In the scheme below, the numbers indicate the number of molecules produced from one of nucleic acid.



Absorption. It is probable that purines are absorbed both free and as nucleosides. It is perhaps significant that nucleosides are more soluble than the sparingly soluble bases of which they are composed. The pyrimidine nucleosides are absorbed as such. These substances are easily absorbed through the blood vessels of the villi. There is no evidence for absorption *viâ* the lymph. Enzymes are available in many tissues for hydrolysing all nucleosides to base and sugar.

Metabolism of Pyrimidines

Normally urine contains no appreciable amount (if any) of pyrimidines. If pyrimidines are ingested the chief end product is urea. They are more readily utilised if administered as nucleosides; cytosine is only appreciably metabolised in this form. The only detailed study of pyrimidine metabolism has been on uracil, which is supposed to be oxidised in five stages to urea and oxalic acid, based on the oxidation of uracil *in vitro*. All four intermediate products are excreted according to expectation. It is sufficient to formulate this oxidation showing only one intermediate product.


Uracil
Formyloxaluric Acid
Urea
Oxalic Acid

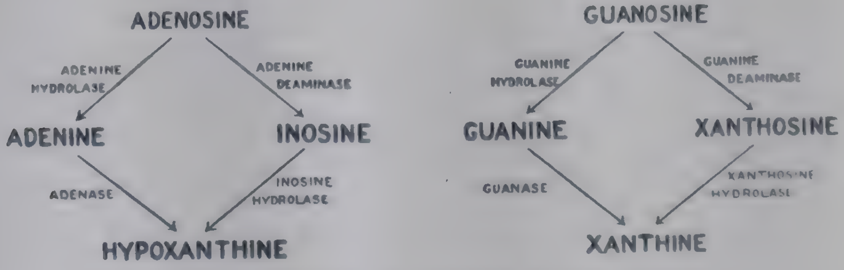
The oxidation is not, however, quantitative.

Pyrimidines are not essential dietary constituents and can be synthesised in the body.

Metabolism of Purines

The metabolism of purines is confused by being partly exogenous and partly endogenous. The end product of purine metabolism in man, **uric acid,** is always excreted even on a purine-free diet or in starvation. The amount excreted (about 0·75 g. per day) under normal conditions is affected by the purine intake, and is roughly halved by omission of purines from the diet.

Katabolism. The stages in the oxidation of purines to uric acid are fairly clear. Purines either free or in the form of nucleosides are deaminised to hypoxanthine (from adenine) or xanthine (from guanine). These reactions can apparently occur in several organs which contain the necessary enzymes. Hypoxanthine may be formed from adenosine either by hydrolysis to adenine and subsequent deamination, or by deamination of adenosine to inosine and subsequent hydrolysis, each step requiring different enzymes. The distribution of enzymes in the tissues decides the path of conversion of adenosine to hypoxanthine. Similarly, alternative paths are available for guanosine. The ultimate result, however, is the same whatever the path followed. This is shown in the following scheme which gives the enzymes concerned at each stage.


ADENOSINE
ADENINE HYDROLASE
ADENINE DEAMINASE
ADENINE
INOSINE
ADENASE
INOSINE HYDROLASE
HYPOXANTHINE
GUANOSINE
GUANINE HYDROLASE
GUANINE DEAMINASE
GUANINE
XANTHOSINE
GUANASE
XANTHOSINE HYDROLASE
XANTHINE

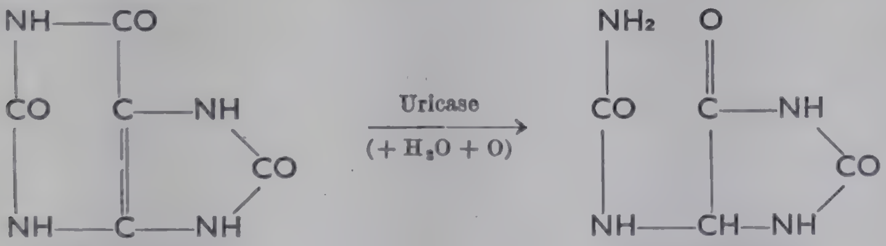
Deamination of purines in the tissues.

The distribution of the enzymes varies not only in different animals, but in different organs in the same animal, so that two paths may be followed in the same animal in different organs. In man, adenase is probably entirely absent ; guanase is present in the kidney, liver and lung, but not in the pancreas or spleen. In other animals (except the pig) guanase is widely distributed, and adenase relatively rare. The two enzymes are rarely found in the same tissue ; ox liver is an exception. The hydrolases and deaminases are almost universally distributed.

The oxidation of hypoxanthine and xanthine to uric acid is confined to the liver, which contains the necessary enzyme, *xanthine-oxidase.* This enzyme is not found elsewhere. It is particularly abundant in the liver of man, but absent from the liver of the dog and rat, our chief experimental animals. The final stages of purine oxidation in man can be represented :—

$$\left.\begin{array}{l}\text{HYPOXANTHINE}\\ \quad\downarrow\\ \text{XANTHINE}\\ \quad\downarrow\\ \text{URIC ACID}\end{array}\right\}\text{Xanthine-oxidase}$$

Man and the anthropoid ape are almost unique in excreting uric acid without further oxidation. Nearly all other animals excrete allantoin along with relatively small quantities of uric acid. This further oxidation of uric acid is catalysed by the enzyme *uricase* which is practically confined to the liver in its distribution. It is absent in man and the ape, but abundant in all other animals.



URIC ACID ALLANTOIN

Since the dog excretes about 98 parts of allantoin to every 2 of uric acid * and most animals over 80 parts of allantoin, there were great difficulties in studying purine metabolism until it was discovered that *pure-bred* * Dalmatian coach-hounds resemble man in excreting uric acid and only relatively little allantoin, even on a purine-free diet.

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* The percentage ratio of allantoin N to allantoin + uric acid N is known as the "*uricolytic index.*" The uricolytic index of man and the chimpanzee is 0, of the pure-bred Dalmatian coach-hound 32, and of other dogs 98. Crosses of Dalmatians with other dogs have the same high index as other dogs.

Exogenous Purine Metabolism. Only about half the ingested purine can be recovered in urine as uric acid. As with the pyrimidines, combined forms of purine are more readily utilised than the free base. Even ingested uric acid is only partially excreted. It is not known whether the retained purine is synthesised to nucleoprotein or broken down by another path.

Endogenous Purine Metabolism. Almost all tissues contain enzymes capable of breaking nucleoprotein down to nucleosides, which could be oxidised to uric acid as outlined above. Endogenous uric acid cannot be regarded as being entirely derived from breakdown of nucleoprotein in view of its ready synthesis. The possibility of its synthesis in mammals from lactic acid and urea, as in birds, has not been excluded. Histidine is another possible source of purine (p. 283).

The increase in excretion of uric acid on a purine-free diet caused by protein is probably due to the specific dynamic action of the protein. Increased tissue activity will explain the increase in uric acid excretion caused by vigorous muscular exercise. As might be expected, conditions involving excessive nuclear breakdown, such as leukæmia and pneumonia, result in markedly increased blood and urine uric acid.

Biochemical Synthesis of Purines. There is no lack of evidence for the formation of nuclear material from non-purine precursors.

(1) The salmon, while migrating from the sea to spawn, converts muscle protein into nucleoprotein of the testes. During the whole of this period (five to fifteen months) the salmon takes no food.

(2) A hen's egg is almost purine-free. During incubation, purines are formed.

(3) Milk is practically purine-free, yet very young mammals fed on it increase their nuclear material.

(4) On low purine diets, which can be continued indefinitely without ill-effects, the purine excretion is greater than the intake.

(5) Benedict kept a Dalmatian coach-hound on a purine-free diet for one year. At least 90 g. of uric acid was formed which could be accounted for in no other way than by synthesis in the animal.

(6) Evidence has been obtained suggesting that rats can synthesise purines from histidine or arginine (p. 283).

Gout

This condition is characterised by the deposition of urates on joint cartilage. It is always associated with a high blood uric acid (up to

9 mg. per 100 c.c.; the normal value is from 0·3 to 4 mg. per 100 c.c.). The high value is probably due to deficient excretion rather than abnormal production of uric acid, although urea is eliminated normally. The deposition in the joints is not caused by the increased blood uric acid alone, for much higher values (and no gout) are observed in leukæmia and nephritis. The cause of the disorder will probably remain obscure as long as we remain in ignorance of the nature of blood "uric acid," since the form in which uric acid circulates in the blood, the extent to which it is combined and the other substances estimated as uric acid in blood have yet to be clearly defined.

CHAPTER XXII

INTERCONVERSIONS OF CARBOHYDRATE, FAT AND PROTEIN (48)

BIOCHEMICAL SYNTHESIS OF CARBOHYDRATE

A. From Fat. The conversion of fat into carbohydrate in the animal is still disputed ; in the plant the conversion is generally accepted. There is no doubt that the glycerol (composing about one-tenth of the fat molecule) forms glucose ; it is the possibility of the fatty acids forming glucose that is disputed. Many experiments claiming the formation of sugar or glycogen from fatty acids (usually by determination of R.Q.) have been reported and as frequently invalidated by cogent criticism. There is as yet no convincing proof of fatty acid conversion in the animal ; but neither can it be said that the possibility has been dismissed. The question of utilisation of fat in muscle has yet to be cleared up (p. 256). The low respiratory quotient of hibernating animals is sometimes cited as a conversion of fat to carbohydrate, but the validity of the methods of determination of the respiratory quotients in these experiments is questionable.

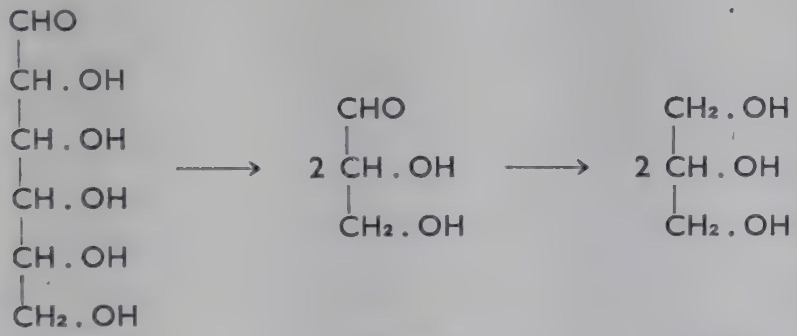
The fatty acids referred to above are those obtained from natural fats, *i.e.*, those with an even number of carbon atoms. Fatty acids with odd numbers of carbon atoms, propionic, valeric, heptoic, and nonylic, do form glycogen in the liver of a starving rat. These fatty acids are not found in natural fats.

B. From Protein. There is overwhelming evidence of the formation of glucose or glycogen from proteins and certain amino-acids, based on the excretion of glucose by starved pancreatectomised or phlorrhizinised animals after administration of these substances. It is usually taken that 60% of food protein can form sugar. The amino-acids forming sugar are listed on p. 272, and the probable mechanisms for the individual amino-acids in Chapter XX. As to the amount of carbohydrate formed from protein under normal conditions and normal feeding it is not easy to speak. The fact that high protein diets can be ingested indefinitely, without any indication of impairment of that part of metabolism for which carbohydrate is essential, is compatible with the idea that carbohydrate may be formed from protein under normal conditions. There is evidence that protein (if in excess) is used as fuel for muscular exercise. It is usually agreed that the conversion of excess protein to carbohydrate is a normal metabolic process.

BIOCHEMICAL SYNTHESIS OF FAT

C. From Carbohydrate. It has long been an undisputed agricultural fact that fat cattle are produced by increasing the carbohydrate in their food. Young pigs fed on barley lay down far more fat than can be accounted for by the fat and protein content of the barley, even if complete conversion of protein to fat were assumed. This is in accord with human experience. Animals can live and grow fat on diets which are practically devoid of fat.

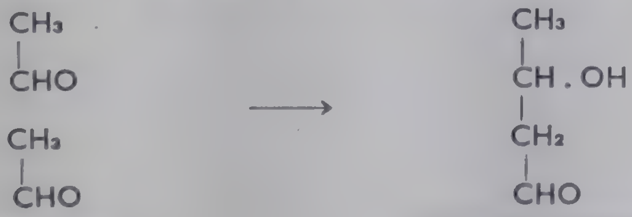
While the fact of the conversion of carbohydrate into fat is amply proven, the details of the change are unknown. The glycerol can be formed from glycerose which is readily formed from glucose.



$$\begin{array}{l}CHO\\ |\\ CH.OH\\ |\\ CH.OH\\ |\\ CH.OH\\ |\\ CH.OH\\ |\\ CH_2.OH\end{array} \longrightarrow 2\begin{array}{l}CHO\\ |\\ CH.OH\\ |\\ CH_2.OH\end{array} \longrightarrow 2\begin{array}{l}CH_2.OH\\ |\\ CH.OH\\ |\\ CH_2.OH\end{array}$$

Glucose Glycerose Glycerol

The production of fatty acid presents greater difficulty. It is usually supposed that acetaldehyde is formed from glucose. Acetaldehyde readily undergoes aldol condensation thus :—



$$\begin{array}{l}CH_3\\ |\\ CHO\\ \\ CH_3\\ |\\ CHO\end{array} \longrightarrow \begin{array}{l}CH_3\\ |\\ CH.OH\\ |\\ CH_2\\ |\\ CHO\end{array}$$

Acetaldehyde Hydroxybutyraldehyde

By reduction of the OH and oxidation of CHO, butyric acid would be formed. Further condensation of butyraldehyde with acetaldehyde would give a six-carbon compound, from which caproic acid could be formed. In this way fatty acids with even numbers of carbon atoms (it will be remembered that those with odd carbon atoms are not found in animals) could be produced. Why acids of sixteen and eighteen carbon atoms, particularly the latter, should predominate, as they do in the animal, is not clear. (These reactions are purely chemical hypotheses which are only supported by absence of any evidence to the contrary.)

D. From Proteins. It has been shown by feeding experiments on depancreatised animals that more than half of the protein molecule can be converted into glucose. Many amino-acids can be shown to give rise to glucose and are antiketogenic. It is therefore to be expected that this carbohydrate is convertible into fat. But the demonstration of the formation of fat from protein is difficult, for the chief effect of increasing the protein ration is to increase the general metabolism (p. 378), so that it is not easy to persuade animals to eat sufficient to provide a surplus. Dogs can be fed for long periods on lean meat without putting on fat. Atkinson, Rapport and Lusk have recently demonstrated by careful quantitative experiments that fat is produced from protein, but only under the abnormal condition of stuffing the dogs with lean meat and preventing their exercise. We can conclude that synthesis of fat from protein is possible, but would not occur under normal conditions.

BIOCHEMICAL SYNTHESIS OF PROTEIN

E. From Carbohydrate. The synthesis of protein from carbohydrate has never been proved. There is evidence of the possibility of the formation of certain amino-acids. Knoop, it will be recalled, studied the deamination of amino-acids by feeding dogs with acids with a phenyl nucleus. The formation of the ketonic acid and ammonia from an amino-acid, of course, suggested the possibility of the reverse action, and he obtained phenyl aminobutyric acid in the urine after feeding the ammonium salt of the corresponding ketonic acid. Embden by liver perfusions with ammonium phenylpyruvate obtained phenylalanine, and alanine from ammonium lactate and pyruvate. Since pyruvic and lactic acids are known to be formed from carbohydrate we can conclude that the formation of alanine from carbohydrate is possible, but that does not mean that other amino-acids can also be formed. The " essential " amino-acids cannot apparently be synthesised by the body even from a diet otherwise complete. For the interconversion of amino-acids see p. 392.

Metabolic studies by Grafe suggested that nitrogen balance could be maintained for a short time on a protein-deficient or even protein-free diet if nitrogen was provided as ammonium citrate, and there was an ample supply of carbohydrate, but it could not be proved that the retained nitrogen had been synthesised to protein. After rats have been fed with ammonium citrate enriched with heavy nitrogen, it can be shown that some N^{15} has been transferred to the amino-acids of the tissues.

Carbohydrate has a greater sparing action on protein than has fat. This has been interpreted as indicating a metabolic association of protein and carbohydrate.

F. From Fat. Such evidence as there is suggests that if protein is formed from nitrogenous sources other than amino-acids, carbohydrate is involved. Of the formation of protein from carbohydrate there is as yet no proof, nor can the formation of carbohydrate from fat be established. Since the conversion of fat into protein would presumably involve intermediate formation of carbohydrate, it is not unreasonable to conclude that fat is not converted into protein.

Summary

Interconversion of	*Conclusion*
C. Carbohydrate to Fat	Proved. Normal.
E. Carbohydrate to Protein	No evidence.
A. Fat to Carbohydrate	Possible, but unproved.
F. Fat to Protein	Improbable.
B. Protein to Carbohydrate	Proved.
D. Protein to Fat	Proved, but only on excessive feeding.

CHAPTER XXIII

CALCIUM AND PHOSPHORUS METABOLISM

(4, 8, 50, 51, 52, 54, 94)

Calcium and phosphorus are the most abundant mineral elements in the body and each has a number of important *rôles*. They are so intimately connected that it is convenient to discuss them together. The chief *rôles* of these elements are :—

Calcium.

(1) Formation of bones and teeth (p. 303).

(2) Blood clotting (p. 151).

(3) Essential for contractility of involuntary (*e.g.*, heart) muscle, acting antagonistically to Na and K.

(4) Influence on excitability of nerve fibres and centres.

(5) Plasma calcium must be maintained near the normal level; a low blood calcium produces tetany (p. 307).

For (3), (4) and (5) see text-books of Physiology.

Phosphorus.

(1) Formation of bones and teeth (p. 303).

(2) Formation of phosphatides essential to every cell (p. 78).

(3) Formation of nucleic acid and derivatives, *e.g.*, adenylic acid (p. 116).

(4) Formation of organic phosphates (of hexose, triose, creatine and adenylic acid) in muscular contraction (p. 237).

(5) Assisting in absorption of glucose (p. 219) and of fats (p. 244).

(6) Formation of phosphatides as an intermediate stage in fat metabolism (p. 250).

(7) Both inorganic and organic (in that at least one acid hydrogen of the phosphoric acid is free) phosphates can take part in buffering in the cells.

(8) While direct buffering in blood by inorganic phosphate is small, the power of the kidney to excrete predominantly acid phosphate from blood which normally contains about 80% alkaline phosphate and only 20% acid phosphate provides a means

of conserving the alkali reserve, since each molecule of Na_2HPO_4 converted into urinary NaH_2PO_4 is, in effect, a molecule of $NaHCO_3$ given to the alkali reserve.

(9) Formation of co-enzymes (p. 129).

(10) Formation of phosphoproteins, *e.g.*, caseinogen in milk.

Phosphorus only occurs in the body in the form of compounds of ortho- and pyro-phosphoric acid.

Sources. Calcium is ingested in the form of inorganic salts and organically bound as in milk. Phosphorus is taken mainly in the form of organic phosphates, *e.g.*, phosphatides, nucleic acid, caseinogen, but also as inorganic phosphate, *e.g.*, in milk and meat. With the exception of phytin (see p. 300), calcium and phosphorus compounds appear to be readily digested to absorbable forms. Mutual reaction, however, prevents complete absorption.

Absorption of Calcium

Absorption of calcium from the intestine is never complete. In an adult in calcium equilibrium about 70% of ingested calcium is ordinarily passed out in the fæces and only 30% in urine; while there is evidence that endogenous (dissolved from bones in starvation) and injected calcium is excreted, like heavy metals, into the large intestine, the amount so eliminated ordinarily is probably small. At least half, therefore, of the ingested calcium can be regarded as normally not entering the body at all. The amount absorbed largely depends upon the nature of the diet, for calcium is only absorbed if it is in a water-soluble form and *if it is not precipitated in the intestine by any other constituent of the diet.* Most foods during digestion liberate phosphates. Now at alkaline or neutral reaction calcium forms an insoluble phosphate which is only soluble at acid reaction. The reaction of the intestine, which is largely determined by the diet, is therefore of great importance for the absorption of calcium. At the normal faintly acid reaction of the intestine calcium phosphate is almost insoluble. Differences in calcium absorption have been shown to occur experimentally in man on a given diet solely by adding hydrochloric acid or sodium bicarbonate. It is, perhaps, significant that calcium is chiefly absorbed from the jejunum where the reaction is most acid. To offset these adverse conditions there are, however, fats and bile salts to facilitate calcium absorption. Bile salts alone have a hydrotropic action (p. 48) on $CaCO_3$ and $Ca_3(PO_4)_2$, but on calcium oleate the effect is at least ten times increased. (A solution containing nearly 8 mg. per 100 c.c. diffusible Ca can be obtained.) This is the probable explanation of the beneficial effect of a *moderate* amount of fat on the absorption of calcium. (An *excess* of fat would use up most of the bile acids for hydrotropic combination with fatty acids.) The beneficial effect of cod-liver oil in this respect is probably due more to the fat it contains than the vitamin D. For the effect of vitamin D on the absorption of Ca and PO_4 see p. 353.

Lactose, owing to formation of lactic *acid* in the intestine, increases the absorption of calcium.

Absorption of Phosphate

The absorption of phosphate is closely related to that of calcium. Normally about one-third of the ingested phosphate (all forms) is passed in fæces and two-thirds in urine. The loss in fæces is mainly due to combination with calcium to form the insoluble phosphate. A high calcium diet or conditions favouring the formation of calcium phosphate in the intestine will diminish phosphate absorption, while moderate amounts of fat or acid assist absorption. Very little phosphate is excreted into the large intestine from the blood.

In most foods the organic phosphate is digested to inorganic phosphate, but in some vegetable foods a large proportion of the total phosphorus is not absorbed. This part of the phosphorus is present in the form of *phytic acid* (inositol hexaphosphate) or *phytin* (CaMg phytate) which can neither be hydrolysed by the enzymes of the digestive juices nor absorbed from the intestine. Owing to the insolubility of its calcium salts phytic acid hinders absorption of calcium. In some cereals, *e.g.*, oatmeal, there is an excess of phytic acid over that required to form an insoluble salt with the calcium present in the cereal, so that the absorption of the calcium of other constituents of the diet besides the oatmeal may be inhibited. It is this prevention of calcium absorption, rather than the unavailability of the phosphorus, which is probably the main cause of the anti-calcifying or rachitogenic action of cereals which contain a relatively large proportion of phytic acid.

It will be realised from the above that the absorption of calcium and phosphorus is by no means a simple process, and that there are many ordinary ways in which it may be hindered or promoted. This is of considerable dietetic importance.

Calcium in Blood

The calcium in blood is entirely confined to the plasma. The erythrocyte membrane is apparently impermeable to calcium. In a given individual in health the amount is extraordinarily constant and lies between 9 and 11 mg. per 100 c.c. plasma (5 and 7 mg. per 100 c.c. blood). The form in which this 10 mg. exists in plasma is still disputed. About 2 mg. is in *ionisable* form ; even this amount would, theoretically, give a supersaturated solution of $Ca_3(PO_4)_2$ in the plasma salts. From 3 to 5 mg. are associated with the proteins in such a way as to be *non-diffusible* through membranes, though whether as calcium proteinate or some physico-chemical complex is unknown. The remainder is in *non-ionised but diffusible* form, the nature of which is disputed.* The calcium and phosphate ions circulating in plasma appear to be in a state of mutual equilibrium analogous to saturation. If one ion is increased, there is a reciprocal diminution of the other, either by excretion or deposition in bone.

Phosphorus in Blood

Phosphorus circulates in the blood in several different forms which are usually grouped under four headings convenient for analysis. These are :—

$$\left.\begin{array}{l}\text{(1) Inorganic phosphate (2–5)}\\ \text{(2) Ester (or “ organic ”) phosphorus (14–29)}\end{array}\right\}\begin{array}{c}\text{“ Acid}\\ \text{soluble}\\ \text{phosphorus.”}\end{array}$$

(3) Lipide phosphorus (or phosphatides) (8–18).
(4) Residual phosphorus. (Nucleic acid.) (Very small.)

The normal values (mg. per 100 c.c.) are given in brackets. The inorganic phosphate is distributed equally between plasma and corpuscles ; the ester phosphorus is predominantly in the corpuscles and includes hexosephosphates, glycerophosphates, and nucleotides ; the lipide phosphorus is higher in the corpuscles and includes lecithin, kephalin, and sphingomyelin. The estimation of inorganic phosphate has received most attention clinically, and is of value in the diagnosis of rickets, where values of about 2 mg. may be observed in *children* (normal 4–6 mg.).

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* Explanations based upon supersaturation, hydrotropy, analogy to calcium citrate, and effect of parathyroid hormone have been put forward ; none have been disproved.

Blood contains phosphatases, which hydrolyse phosphate esters to inorganic phosphate, mainly in the red cells. Special care must be taken to prevent formation of inorganic phosphate by the enzymes in blood samples taken for analysis.

Composition of Bone

Bone consists of an organic matrix upon which insoluble inorganic salts have been deposited ; in this way great rigidity and mechanical strength are obtained. (The tensile strength of a dog's femur is greater than that of cast-iron.) The intimate admixture of the inorganic and organic parts of bone are readily appreciated if a bone is (*a*) carefully calcined to burn away all organic matter, and (*b*) immersed in dilute hydrochloric acid for several days to remove all inorganic matter. In each case the original shape of the bone is accurately retained, the one being brittle and whiter, and the other flexible and less white.

The **organic matrix** consists of a mixture of proteins, a scleroprotein, *ossein*, a mucoprotein, *osseomucoid*, and a keratin, *osseoalbuminoid*. Ossein, the chief protein of bone, is very similar to, if not identical with, the collagen of cartilage. Cartilage also contains a mucoprotein, chrondromucoid, similar to osseomucoid, and a keratin-like chrondroalbuminoid similar to osseoalbuminoid. These cartilage proteins have already been described (p. 182). The **inorganic part** of bone consists mainly of calcium phosphate and carbonate with small amounts of magnesium phosphate and traces of Na, K, Cl, F, Li, Sr. Typical values for the chief mineral constituents are :—

	Percentage of total inorganic matter
$Ca_3(PO_4)_2$	85
$CaCO_3$	12
$Mg_3(PO_4)_2$	1·5

The exact nature of the mineral part of bone has not been satisfactorily established. Most evidence points to the final form of the calcium in bone as compounds of the type $3Ca_3(PO_4)_2 . CaCO_3$, characteristic of the *apatite* series of minerals, in which $CaCO_3$ may be replaced by $Ca(OH)_2$ $CaCl_2$ or CaF_2.

This structure is in agreement with chemical analysis, refractive index and crystalline structure as revealed by X-rays, but not with all experimental facts. The Ca/P ratio of mammalian bone is extraordinarily constant (1·99–2·04. The ratio for the formula given above is 2·15; the presence of magnesium phosphate would lower the ratio), and suggests that a definite type of compound is deposited. It is by no means generally agreed that the final form of calcium in bone is the form in which it is first deposited; some workers hold that $CaHPO_4$ is first formed and subsequently converted into some compound of $Ca_3(PO_4)_2$. The fact that no $CaHPO_4$ or free $CaCO_3$ can be detected in bone by the time it has been separated for analysis does not necessarily disprove this view.

The actual composition of individual bones is very variable, as would be expected, when their histological structure is considered (*e.g.*, relative amounts of compact and spongy bone). A representative figure for the water content is about 25%; of the dried, marrow-free bone about 60% is mineral matter and 40% protein.

Composition of Teeth

The composition of teeth differs from that of bone quantitatively rather than qualitatively. Teeth are more highly mineralised. The **cementum** is very similar to bone. **Dentine** differs chiefly in its water content, which is not more than 10%, and in a slightly higher mineral content. Both contain an ossein-like protein, yielding gelatin on boiling. **Enamel,** which is the hardest tissue in the body, only contains 5% of water and 4% of protein. The rest is mineral matter, predominantly $Ca_3(PO_4)_2$. The $CaCO_3$ content is much smaller than in bone. The protein is keratin-like and does not yield gelatin on boiling. The composition of enamel, on the one hand, and dentine and cementum on the other, is in accord with their different origins—ectodermal and mesodermal.

Calcification of Bones and Teeth

The process of deposition of calcium salts in bone is a complex one which is only little understood. The inaccessibility of the bones makes them a difficult subject for experimental investigation, which is further hindered by the imperfect intestinal absorption of calcium and phosphate. A number of factors which influence the formation of bones and teeth have been revealed; they include :—

(*a*) The intake of calcium and phosphate, which is dependent upon effective acidity or alkalinity, the fat content of food and the intestinal reaction (see p. 299), as well as vitamin D.
(*b*) The ratio of calcium ions to inorganic phosphate ions in plasma (below).
(*c*) The acid base equilibrium of the blood since it affects (*b*).
(*d*) Magnesium in blood (p. 305, 309).
(*e*) Phosphatase (below).
(*f*) Vitamins D and C (pp. 353, 364).
(*g*) Parathyroid hormone (p. 307).

Chemistry of Calcification. The bones must not be considered as static deposits of calcium phosphate; bones are highly mineralised but *living* tissues in dynamic equilibrium with the tissue fluids. Bone laid down can certainly be redissolved in disease; it is highly probable that it is being continually deposited and redissolved in the normal adult. Studies in this field have been almost entirely confined to calcium phosphate owing to ignorance of the true nature of the material laid down.

An essential condition of any theory of calcification is that it should provide a satisfactory explanation of the deposition of calcium phosphate in the ossifying zones *only*. Several theories which have been propounded to explain the deposition of the salt fail to explain the existence of uncalcified cartilage or even blood vessels. The only theory specifically providing for local calcification is Robison's. According to Robison, osteoblasts (or odontoblasts) and cells in the ossifying zone contain or can secrete an *enzyme* (phosphatase, optimum pH 9) which hydrolyses certain phosphoric esters brought to the ossifying zone by the blood. and so causes a *local* increase in PO_4''' ions.

The precipitation of calcium phosphate would follow the law of mass action

$$\begin{aligned}[Ca^{\cdot\cdot}]^3 \times [PO_4''']^2 &= k\,[Ca_3(PO_4)_2] \\ &= \text{K, the solubility product,}\end{aligned}$$

since plasma can be taken as saturated with respect to $Ca_3(PO_4)_2$. In blood the ion product closely approaches K, so that a local increase in concentration of one ion will cause deposition of $Ca_3(PO_4)_2$ in that region.

In support of Robison's theory, split bones *in vitro* calcify at the ossifying zone if immersed in solutions of calcium glycero- or hexose-monophosphate, but not in solutions of calcium chloride and sodium phosphate. Phosphatase, or the "**bone enzyme,**" as it is called, appears in ossifying zones coincident with the beginning of ossification, and is more abundant in these zones than elsewhere. The teeth of young animals exhibit a high phosphatase activity. Non-ossifying cartilage, *e.g.*, trachea, shows no phosphatase activity. In generalised bone diseases (*e.g.*, osteitis deformans, generalised osteitis fibrosa, osteomalacia and active rickets) the plasma phosphatase rises from the normal 0·1–0·2 Kay units up to 3, suggestive of a leak of phosphatase from the bones, since the increase is roughly proportional to the severity; on treatment the value falls as the condition improves. It is in accord with Robison's theory that other metals giving insoluble phosphates, *e.g.*, Pb, Sr, Li, Ag, Ra, are deposited in bone if administered, and are dissolved from the bone under the same conditions as Ca.

There are, however, several facts which suggest that Robison's theory is incomplete. Several non-ossifying tissues (kidney, intestine, lung) have a considerable phosphatase content ; it is perhaps significant that these tissues are known to calcify under pathological conditions. Rachitic bones are not noticeably deficient in phosphatase. Excess of magnesium ions has an adverse effect on calcification ; fluorides may also interfere with calcification, especially in teeth, 1·3 parts per million in drinking water causing "*mottling*" of the enamel.

The bone enzyme only attacks monophosphoric esters. In blood about 95% of such esters are found in the red cells which also contain a phosphatase. The content of these esters in the red cells is raised during bone growth and lowered in rickets. It is not yet clear whether the ester for calcification is the small amount in plasma or that in the red cells, and, if the latter, how it gets out, for if the cell was freely permeable to the ester a more equal distribution between cells and plasma would be expected.

Decalcification of Bone

There is ample evidence that calcium or other metals deposited in bone can be subsequently removed experimentally. Administration of parathyroid hormone, or the production of acidosis with ammonium chloride, causes liberation of calcium from the bone and its excretion in urine. Lead administered to cats has been shown to be deposited in their bones and has subsequently been completely removed therefrom by treatment with parathyroid hormone. Elimination of calcium in urine and fæces does not cease when intake is diminished or stopped; in prolonged starvation the plasma calcium is not lowered, calcium being liberated from the bones owing to the starvation acidosis. There is probably continual wear and replacement of bones just as of tissue proteins, and some maintain that the ease of decalcification is such that the bones form an additional alkali reserve. This dynamic nature of bone formation has been confirmed recently by experiments with radioactive phosphate. Bones are especially liable to decalcification when there is a dietary deficiency (of Ca, PO_4 and vitamin D) and at the same time an extra demand for calcium, as in pregnancy; this is the cause of the osteomalacia (adult rickets) chiefly observed in malnourished pregnant women. In several pathological conditions the bones are partly decalcified; this loss of mineral matter is replaced on recovery. Bone is more easily decalcified than teeth. Extensive bone decalcification can occur without evidence of dental caries.

Decalcification of Teeth. Dental Caries (83)

Although dentine is less liable to decalcification by the factors mentioned, the incidence of dental caries is very high even in the apparently well-nourished. In South Africa, for example, where rickets is rare, caries is prevalent. It must be concluded that the mechanisms involved are different. Teeth, unlike bone, are exposed to external influences such as saliva and the bacteria of the mouth. Caries is apparently unrelated to the Ca, P, or CO_2 content or alkalinity of the saliva or the Ca or P content of plasma. Numerous attempts to correlate its incidence with the vitamin D of the diet have not given conclusive results. The general impression of increased susceptibility to caries in pregnancy and lactation is not supported by controlled dietary experiments on large groups of women. Another alleged cause of dental caries is formation of acid from food residues by bacterial action in the mouth; carbohydrate especially has come under suspicion in this respect. Yet in Tristan da Cunha, where the inhabitants had a high carbohydrate (potato) intake, caries was rare. Other causes which have received attention are heredity, purified sugar, white bread, soft food, deficiency of vitamin C, general dietetic habits. None has provided a convincing explanation. The impression is left that an hereditary susceptibility to caries may be greatly magnified by dietetic errors. It is, perhaps, worthy of note that in a large group of children recently selected for their freedom from caries all were found to have had a preponderance of raw fruits and vegetables in their diets.

Control of Calcium Metabolism by the Parathyroid Hormone

The plasma calcium level (normally 10 mg. per 100 c.c.) appears to be controlled by the parathyroid hormone. If the parathyroids are removed, the plasma calcium level falls below 7 mg. per 100 c.c. (and phosphate rises correspondingly) and tetany ensues. The signs of tetany (spasmophilia) are increased excitability of peripheral nerves resulting in characteristic spasms of the limbs supposed to be due to a deficiency of calcium ions in the fluid bathing the nerves and muscles.* The condition is relieved by administration of calcium or by injection of the parathyroid hormone. Hypersecretion of parathyroid hormone or excessive administration leads to a raised blood calcium (up to 20 mg. per 100 c.c.) with reciprocal lowering of phosphate. This calcium is obtained at the expense of the bone, since hyperparathyroidism causes bone diseases such as generalised osteitis fibrosa. Excessive administration causes increased excretion of Ca or any similar metal deposited in bone (p. 306).

How the parathyroid maintains a constant blood calcium level is far from clear. Its action is probably confined to the diffusible forms of calcium, since tetany does not ensue when the non-diffusible calcium only is reduced, as in nephritis. There is little doubt that the hormone can decalcify bones directly. It is responsible for preventing the normal calcium from leaving the blood; this has been expressed as the power of the hormone to increase the solubility of diffusible calcium in the blood. It will be recalled that the calcium and phosphate in the blood are inversely reflected in each other ; this has led some to the opinion that the parathyroid primarily affects phosphate, which in turn affects the calcium. In support of this view evidence is accumulating that the parathyroid hormone acts upon the phosphate-secretory mechanism of the kidney.

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* Other forms of tetany, such as those seen in rickets, osteomalacia, severe nephritis and alkalæmia (*e.g.*, from hyperpnœa or excessive ingestion of $NaHCO_3$), can be ascribed to the same reason.

For the nutritional requirements of Ca and P, see pp. 396–397.

CHAPTER XXIV

MINERAL METABOLISM (4, 8)

Many of the mineral elements found in the body are essential to health. In many cases our knowledge of their functions is not very extensive. The metabolism of Ca and P has been dealt with in the previous chapter. The nutritional requirements of mineral elements are discussed on pp. 396–398.

Magnesium (52, 82)

About 70% of the magnesium in the body is present as phosphate in bone and probably deposited there under similar conditions to calcium. Voluntary muscle contains about 0·02% of magnesium (calcium = 0·007%). In that it forms insoluble phosphates magnesium is absorbed in much the same way as calcium (p. 299). Its distribution in blood differs from calcium in that it is fairly equally shared between corpuscles (2–4 mg.) and plasma (1–4 mg. per 100 c.c.). Under normal conditions the magnesium intake is always adequate, so that effects comparable to calcium deficiency are not seen. Experimental deprivation of magnesium leads to a type of tetany, not identical with calcium tetany, which is fatal in rats after about eighteen days. The serum magnesium is lowered and the blood cholesterol increased while the percentage of calcium in soft tissues, especially kidneys and muscles, is raised even to the extent of deposition of calcium. The effects of magnesium deficiency are more severe if the calcium intake is increased. Other observations also suggest an interrelationship between the metabolisms of calcium and magnesium. For example, where there is decalcification of the skeleton, as in rickets or osteomalacia, the magnesium content of the bones is higher than normal; carious teeth contain more magnesium than sound teeth. There is as yet no explanation of this interrelationship.

An important *rôle* of magnesium ions is the activation of several enzymes, especially phosphatases.

Sodium

In man sodium salts are predominantly distributed in the body fluids, whereas potassium is mainly intracellular. The reason for this uneven distribution of readily diffusible ions is unknown. The sodium of the body in combination with chlorine largely regulates the osmotic pressure of the body fluids (p. 26). The sodium in plasma provides over 90% of the total base. Sodium bicarbonate and sodium phosphate are important blood buffers. Sodium chloride is probably the chief source of the HCl of the gastric juice. Sodium ions are essential for the contraction of involuntary muscle. The excretion of sodium, which is predominantly by the kidney, is diminished if the intake is deficient. In animals, continued deprivation over long periods is ultimately fatal. Animals have a very strong instinct for salt (p. 385), so that natural deficiency is rare. Sodium cannot be replaced by potassium and *vice versâ.* For the influence of the suprarenals on sodium metabolism, see p. 334.

Potassium (90)

Potassium salts contribute to the regulation of osmotic pressure and buffering. The salts concerned in buffering the red cell are the hæmoglobinate, bicarbonate and phosphate. A proper balance between potassium and calcium is essential to the contraction of heart muscle. Like sodium, potassium is mainly excreted in urine and retained if the intake is deficient. In experimental animals deprivation of potassium checks growth and is ultimately fatal. For the influence of the suprarenals on potassium metabolism, see p. 334.

Iron

Iron, especially in ionisable forms, is fairly readily absorbed. Animal experiments suggest that any excess iron absorbed is very rapidly excreted, not by the kidneys, but by the large intestine and the bile ; this excretion is so rapid as to account for earlier conclusions that iron, especially ionised iron, was not readily absorbed, since the apparently unabsorbed iron had, in fact, been absorbed and excreted again into the intestine. Recent experiments of Widdowson and McCance, however, suggest that in man excess of iron absorbed is retained and not readily excreted into the intestine. Ferric iron is probably reduced to ferrous form prior to absorption. The greater part of the iron in the body is combined in the hæm of hæmoglobin and is, therefore, essentially concerned in oxygen carriage. The iron normally liberated by breakdown of the hæmoglobin of disintegrated red cells is not excreted, but stored as hæmosiderin (p. 174), and subsequently re-utilised. The daily requirement of iron is, therefore, small and probably in the region of a few milligrams. A deficient intake of iron leads to nutritional anæmia which cannot, however, be cured by administration of pure iron salts, which are only effective if minute amounts of copper are also present. In practice, ordinary iron salts are effective, since the traces of copper present are very difficult to remove. Copper is apparently essential for the utilisation of iron in the formation of hæmoglobin. Excessive ingestion of iron does not cause an appreciable increase in the hæmoglobin content of the blood of a healthy subject.

The exact nature of the traces of iron in plasma is unknown. Iron is probably an important intracellular catalyst of oxidation-reduction reactions.

Copper

Little is known of the metabolism of copper except that it is important in nutritional anæmia (see iron above). It is, perhaps, significant that the copper content of the brain and liver of the fœtus and infant is greater than that of the adult. (The fœtus stores up iron in the liver which tides the infant over the normal period of suckling, for milk contains insufficient iron and copper for its needs.) The total amount of copper in the adult is about 0·1 gram. It is chiefly present as copper protein complexes, notably in certain oxidases, *e.g.*, cytochrome oxidase, polyphenol-oxidase and ascorbic acid oxidase. A blue copper protein, hæmocuprein, accounts for the copper in the erythrocyte. A similar protein, hepatocuprein, occurs in liver. A deficiency of copper has been shown to be responsible for several diseases of farm animals.

Manganese

This element is widely distributed in the body, especially along the alimentary canal and in the liver and reproductive organs. Experimental deficiency of manganese in the diets of rats leads to disturbance of the œstrus cycle and lactation in the female, and to degeneration of germinal epithelium in the male.

Zinc

Zinc is an indispensable element. In practice a deficiency is unlikely to occur owing to the widespread distribution of the element in foodstuffs. Zinc forms part of the enzyme carbonic anhydrase which is concerned in the transport and excretion of carbon dioxide (p. 326) A small amount of a zinc salt prolongs the action of insulin (p. 232) and zinc is essential for the crystallisation of insulin (p. 335). The alleged correlation between the incidence of tuberculosis and cancer and the geographical distribution of zinc has not been proved.

Aluminium

There is no evidence that aluminium is an essential element. The widespread use of aluminium cooking utensils has raised the question of the effect of the small quantities of the metal dissolved by the food cooked in them. While the point is still contested, there is as yet no convincing reason for believing that even greater quantities of aluminium are harmful to man.

Other Metals

Many other metals are present in minute traces in the body. These traces are inevitably present in ordinary foods and there is, as yet, no proof that they have any metabolic function. There is some evidence that traces of cobalt are essential for cattle and sheep, and traces of manganese are essential for birds.

Chlorine

Undoubtedly the major part of the chlorine in the body is present in inorganic form ; the existence of chlorine in organic form (lipides with Cl replacing OH in glycerol) has been suggested. but not convincingly proved. In blood, chlorine ions are distributed between cells and plasma according to the CO_2 tension. The free passage of Cl', but not $Na^{\cdot}$ or $K^{\cdot}$, through the red cell membrane gives rise to Donnan equilibria and the Hamburger phenomenon (chloride shift) described on pages 40 and 327. Chloride provides about two-thirds of the acid of plasma. Chlorides, mainly NaCl, are largely concerned with the regulation of the osmotic pressure and water content of the body (p. 26). The provision of HCl for gastric juice is discussed on p. 191. Chloride is very readily absorbed and normally from 10 to 15 g.

are excreted in urine per day ; in starvation, chlorides are retained, and less than 1 g. per day may be excreted, the blood chlorides remaining normal. Chloride cannot be replaced by other halides.

Iodine

Iodine is important in that it is an essential element in the molecule of the hormone thyroxine (p. 336). In man the thyroid gland contains over half the total iodine (about 25 mg.) of the body. Iodides are readily absorbed and excreted (chiefly in urine). The iodine content of blood is very small (about 0·01 mg. per 100 c.c.), and only partly in ionised form. A deficiency of iodine in the diet causes simple colloid goitre (p. 337).

Bromine

Bromine is distributed throughout the tissues and in blood in greater concentration than iodine. Its function is unknown.

Fluorine

Fluorine is also distributed throughout the tissues in small quantities. It is present as CaF_2 in bone, but does not appear to be an essential constituent. Excessive intake of fluoride (*e.g.*, if the water supply contains more than 1·3 parts per million) causes mottling of the enamel of teeth (*fluorosis*). It has been claimed that teeth mottled by fluorosis are less liable to caries than are unmottled teeth.

Sulphur

Sulphur is ingested very largely in the form of the amino-acids cysteine, cystine and methionine in proteins (food proteins contain on the average 1% of sulphur) ; small amounts are obtained from mucoproteins (as sulphate) and sulphatides. The amount of inorganic sulphate ingested is very small. For details of the metabolism of sulphur compounds, see pp. 266, 276–278. The final oxidation product of sulphur compounds in the body is sulphuric acid, which is excreted as inorganic and ethereal sulphate (p. 452).

Sulphur compounds found in the body and not mentioned above include the *keratins* (pp. 97, 108, 182, 184, 186), *insulin* (p. 335), *glutathione* (p. 143), *vitamin* B_1 (p. 359), *taurine* (pp. 196, 276), *taurocholic acid* (p. 196), *thioneine* (p. 283), and thiocyanates (pp. 191, 318).

CHAPTER XXV

DETOXICATION (4)

We saw in Chapter XIV that certain toxic substances such as phenols were produced by bacteria in the large intestine, and that some were absorbed. These substances are usually rendered less toxic before they are excreted by the kidney. Other poisonous substances may get into the blood stream as a result of ordinary katabolism (*e.g.*, benzoic acid), by being accidentally swallowed or by absorption from the skin. The process by which the body combats chemical poisons is usually referred to as *detoxication*. Substances are detoxicated in various ways and often the same substance is eliminated by more than one path. The methods vary greatly in different animals, and where two methods are used their relative extents may depend upon the diet. The most common detoxicating mechanisms are *oxidation*, *reduction* and *conjugation* (synthesis). Certain amino-compounds may be deaminised, *e.g.*, tyramine forms *p*-hydroxyphenylacetic acid.

Oxidation

The first attempt of the body at removal of a toxic substance is to destroy it by complete oxidation. In the case of aliphatic compounds this is relatively easy, but compounds with aromatic nuclei are not readily oxidised completely. Detoxication of such compounds is often achieved by oxidative formation of groups which can be used for detoxication by conjugation, *e.g.*, a phenolic hydroxyl group which can be conjugated with glucuronic or sulphuric acids, or a carboxyl group (by oxidation of an aliphatic side chain) which can be conjugated with glycine or glucuronic acid. Indole produced by bacterial digestion of tryptophan is oxidised to indoxyl prior to conjugation.

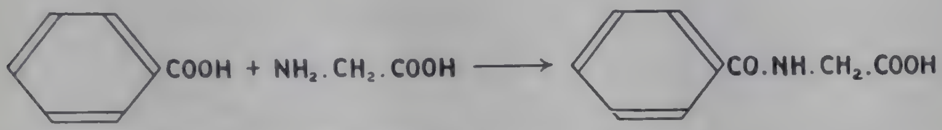
Reduction

An example of reductive detoxication is the conversion of aromatic nitro-compounds to amino-compounds, which can then be detoxicated by conjugation. *m*-Nitrobenzoic acid is reduced to *m*-aminobenzoic acid, picric acid, $C_6H_2(NO_2)_3OH$, to picramic acid, $C_6H_2(NO_2)_2(NH_2)OH$, and chloral, $CCl_3 . CHO$, to $CCl_3 . CH_2OH$.

Conjugation

Failing destruction by oxidation or reduction, toxic substances are combined with other compounds to form less toxic and (usually) more soluble compounds. The chief substances used for conjugation in this way are glycine, glutamine, glucuronic acid, sulphuric acid, and acetic acid.

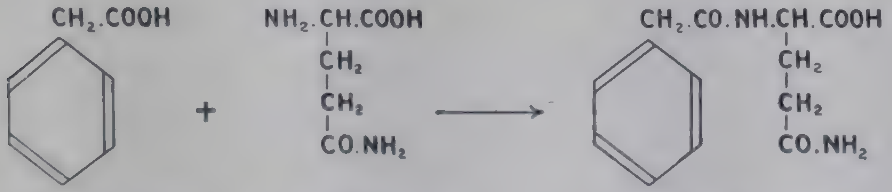
Glycine. Glycine is one of the most extensively used substances for detoxication. A very large number of aromatic acids have been shown to be detoxicated by it. The best known and commonest glycine conjugation product is hippuric acid, formed from benzoic acid.



BENZOIC ACID GLYCINE HIPPURIC ACID
(Benzoylglycine)

Benzoic acid is detoxicated in this way by all mammals, and hippuric acid is a normal constituent of urine. The glycine for conjugation can be readily synthesised in the body in its absence from the food, and apparently at the expense of waste nitrogen rather than the tissues, since increased detoxication of benzoic acid corresponds with a decrease in the excretion of urea.

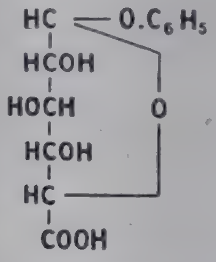
Glutamine. Glutamine is used by *man* for detoxication of phenylacetic acid. Most other mammals use glycine, forming phenylaceturic acid (p. 252). Phenylacetyl-glutamine is only formed by man and the chimpanzee. Derivatives of phenylacetic are not, however, detoxicated by glutamine in man.



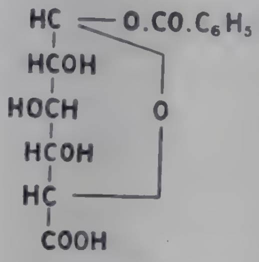
PHENYLACETIC ACID GLUTAMINE PHENYLACETYL GLUTAMINE

Glutamine can be formed at the expense of waste nitrogen, like glycine. In the fowl phenylacetic acid is detoxicated by ornithine, also formed at the expense of waste nitrogen.

Glucuronic Acid. Conjugation with glucuronic acid occurs in all species. This acid can combine with many substances which contain (or form by oxidation or reduction) hydroxyl groups (alcoholic or phenolic) as well as with acids like benzoic or phenylacetic. Examples of these two types of compound are phenylglucuronic acid and benzoylglucuronic acid.



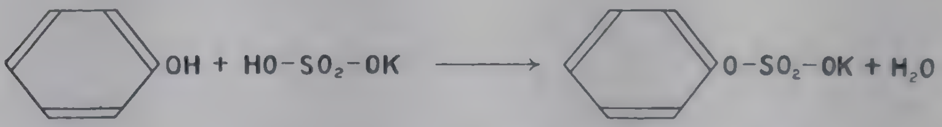
GLUCOSIDE TYPE
(Phenylglucuronic Acid)



ESTER TYPE
(Benzoylglucuronic Acid)

Glucuronic acid is generally supposed to arise from glucose (or glucogenetic amino-acids), although there is, as yet, no clue to the mechanism; the conjugation takes place in the liver. Glucuronic acid plays a large part in detoxication, since it can conjugate with the compounds detoxicated by glycine or sulphuric acid.

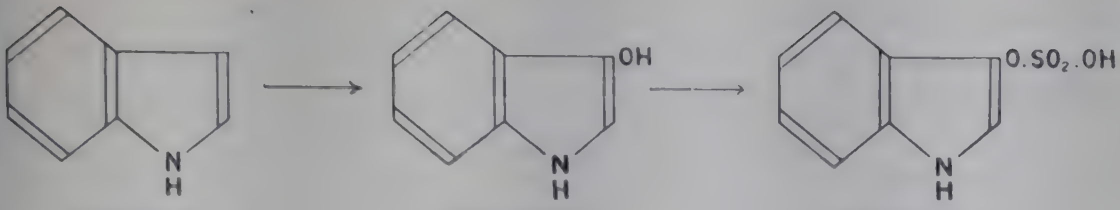
Sulphuric Acid. Another important detoxication mechanism is the combination of phenols with sulphuric acid to form sulphuric esters or **ethereal sulphates.**



PHENOL

POTASSIUM PHENYL
SULPHATE

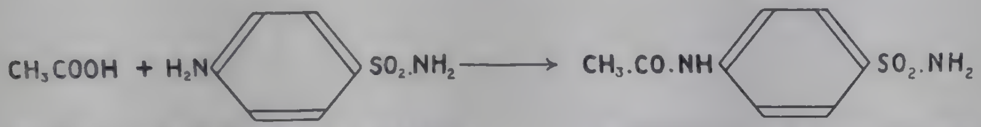
The ethereal sulphate usually exists as a salt of Na or K. Whether the ethereal sulphate is formed direct from inorganic sulphate or by oxidation of a conjugation product of the phenol with some other sulphur compound is not certain. The ethereal sulphate of normal urine is derived partly from phenols absorbed from the large intestine (p. 201). It will be remembered that indole and skatole are also absorbed. Indole is oxidised shortly after absorption to indoxyl and then conjugated with sulphuric acid.



INDOLE INDOXYL INDICAN

The ethereal sulphate of indoxyl is known as **indican.*** Skatole is said to be oxidised to skatoxyl, a compound whose structure is unknown (if it exists).

Acetic Acid. Acetic acid is used for the detoxication of amino compounds and forms an N-acetyl compound. Acetic acid would be readily available by breakdown of fats or carbohydrate. A typical example is the detoxication of sulphanilamide.



ACETIC ACID SULPHANILAMIDE ACETYLSULPHANILAMIDE

Acetic acid does not appear to be used to detoxicate OH groups.

The common conjugations in man can be summarised :—

Compound Detoxicated	*Conjugating Compound*	
Alcoholic	Glucuronic Acid.	
Aldehydic or Ketonic (after reduction)	Glucuronic Acid.	
Aromatic Amino	Acetic Acid.	
Benzoic Acid	Glucuronic Acid.	Glycine.
Phenylacetic Acid	Glucuronic Acid.	Glutamine.
Phenolic	Glucuronic Acid.	Sulphuric Acid.

Note the predominance of glucuronic acid. All available methods of detoxication are usually employed, *e.g.*, sulphanilamide is detoxicated by acetylation of the amino group and by formation of glucuronide and ethereal sulphate from an —OH introduced by oxidation. As to the site of detoxication, the main one is undoubtedly the liver. Some of these reactions have, in fact, formed the bases of tests of liver efficiency, *e.g.*, the rate of formation of hippuric acid from ingested benzoic acid. The rate of elimination of hippuric acid may, however, be affected by impaired renal function.

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* Often called *urinary indican* to distinguish it from the indican of the indigo and woad plants, which is a *glucoside* of indoxyl. Urinary indican is frequently formulated as the potassium salt, although there seems to be no particular reason for so doing.

It is perhaps significant, as Quick observed, that in most instances of detoxication the result has been to form a substance more strongly acidic than the original one, for it will be recalled that the body is particularly well protected against excess acidity. This formation of acid products is presumably to ensure their rapid elimination by the kidney.

Other Mechanisms. Small amounts of *cyanides* are supposed to be detoxicated by conversion to the relatively harmless thiocyanates which are excreted by the kidneys and some of the digestive juices, *e.g.*, saliva. In dogs cysteine detoxicates *bromobenzene* as a mercapturic acid ; *pyridine* and *nicotinic acid* are methylated. Rats can demethylate certain aromatic methylamino compounds.

CHAPTER XXVI

THE UTILISATION OF OXYGEN AND EXCRETION OF CARBON DIOXIDE (3, 4, 8, 61)

In the chapters on the metabolism of the carbon compounds it has frequently been stated that certain substances are oxidised. These oxidations are ultimately effected by the oxygen obtained from the air during respiration and it is the purpose of this chapter to show how this oxygen is brought to the tissues in which it is used.* Briefly, some of the oxygen inspired into the lungs combines with the hæmoglobin of the blood, thereby converting it almost entirely into oxyhæmoglobin. This passes to the tissues where some of the oxyhæmoglobin yields up its oxygen to the tissue cells ; the partly deoxygenated hæmoglobin then travels back to the lungs where it is reoxygenated. In using oxygen the tissues produce carbon dioxide which is taken up by the blood, carried to the lungs and excreted in the expired air.

The average oxygen consumption of a man during working hours is about 400 c.c. per minute. The use of this oxygen involves the formation of considerable amounts (340 c.c. if the R.Q. is 0·85) of carbon dioxide from the carbon in the substances oxidised. That this carbon dioxide is removed by way of the lungs is evident from a comparison of the compositions of inspired and expired air. The figures given below are volumes per cent. and refer to dry gases at N.T.P.

	Inspired Air	*Expired Air*	*Alveolar Air*
Oxygen . .	20·93	16·93	14·08
Nitrogen . .	79·04	79·55	80·37
Carbon dioxide .	0·03	3·52	5·55

There is a small difference in the *percentage* of nitrogen because the total volume of expired air is usually less than that of inspired air. The *volume* of nitrogen expired is actually the same as that inspired. The total volume loss is due to more oxygen being used than carbon dioxide produced, since the respiratory quotient is less than 1 ; in the example given it is 0·85.

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* The manner in which the respiratory movements are controlled and abnormalities of respiration are beyond the scope of this book. For these the reader must consult Textbooks of Physiology.

There is a greater difference between the compositions of inspired air and the air which is in contact with the alveoli—**alveolar air.** Expired air is a mixture of alveolar air with the unchanged inspired air required to fill the " dead space."

The removal of carbon dioxide from the tissues by the blood is revealed by analyses of the gases contained in arterial and venous blood. The values below are c.c. per 100 c.c. blood.

	Oxygen	*Nitrogen*	*Carbon Dioxide*
Arterial blood .	19	1	50
Venous blood .	13	1	58

These gases are given off if the blood is exposed to a vacuum, although oxygen is more conveniently estimated by Haldane's method, using potassium ferricyanide according to the equation on p. 167 and carbon dioxide by expelling by means of acid as in Van Slyke's method. (For details of these methods the reader should consult Refs. 61 or 8.)

It is now necessary to define certain terms used in discussing the blood gases.

The **Oxygen Capacity** of blood is the number of c.c. of oxygen which 100 c.c. of whole blood contain when fully saturated with air. It comprises oxygen bound by hæmoglobin and oxygen in physical solution.

The **Oxygen Combining Power** is the number of c.c. oxygen combined with hæmoglobin in 100 c.c. of the fully saturated blood, *i.e.*, it is the *Oxygen Capacity less the Oxygen in physical solution.* Since 1 c.c. oxygen combines with 0·746 g. hæmoglobin a determination of the oxygen combining power will enable the hæmoglobin content of the blood to be calculated.

The **Oxygen Content** is the number of c.c. oxygen in 100 c.c. of the blood which must, of course, be collected without loss of gases or exposure to air, *e.g.*, under paraffin.

The **Percentage Oxygen Saturation** is the amount of oxygen combined with hæmoglobin in 100 c.c. of the blood expressed as a percentage of the combining power of that blood, *i.e.*,

$$\frac{\text{Oxygen content} - \text{c.c. oxygen in physical solution}}{\text{Oxygen capacity} - 0{\cdot}4} \times 100$$

The oxygen in physical solution in blood fully saturated in air is about 0·4 c.c., in arterial blood about 0·2 c.c and in venous blood about 0·1 c.c. These corrections are often neglected.

The **Oxygen Unsaturation** = 1·00 — the Oxygen Saturation.

The **Carbon Dioxide Capacity** is the number of c.c. carbon dioxide in combination plus the carbon dioxide in physical solution in 100 c.c. of blood equilibrated with carbon dioxide at 40 mm. partial pressure.

The **Carbon Dioxide Combining Power** is the carbon dioxide capacity less the carbon dioxide in physical solution.

The **Carbon Dioxide Content** is the number of c.c. carbon dioxide in 100 c.c. of the blood which must, of course, be collected without loss of gases or exposure to air.

The **Alkali Reserve** or **Plasma Bicarbonate** is the volume of carbon dioxide which is expelled by acid from 100 volumes of plasma which has previously been equilibrated (usually at room temperature) with alveolar air containing carbon dioxide at 40 mm. tension, the carbon dioxide in physical solution being deducted.

Gas volumes in the above are measured dry at N.T.P.

THE CARRIAGE OF OXYGEN BY THE BLOOD

Oxygen is not carried in the blood by mere solution in the plasma. If a gas is in simple solution in a liquid it is dissolved in an amount varying directly with the partial pressure of the gas. 100 c.c. of water at 37° C. exposed to pure oxygen at 760 mm. dissolves 2·42 c.c. If the oxygen pressure be doubled, *i.e.*, 1,520 mm., then twice the amount of oxygen will be dissolved, *i.e.*, 4·84 c.c. In a mixture of gases, provided that there is no chemical interaction, the amounts of each gas dissolved are proportional to the partial pressures of the respective gases and independent of the nature of the gases. Air contains approximately 4 parts nitrogen and 1 part oxygen, so that at 760 mm. the partial pressure of nitrogen is $4/5 \times 760 = 608$ mm. and that of oxygen is $1/5 \times 760 = 152$ mm. Therefore, since the amount of oxygen dissolved varies as its partial pressure 100 c.c. of water at 37° C. exposed to air at 760 mm. will dissolve $1/5 \times 2{\cdot}42 = 0{\cdot}48$ c.c. oxygen. If the pressure is reduced by half to 380 mm. then oxygen will come off until equilibrium is established with 0·24 c.c. oxygen in solution. If water contains dissolved substances gases are usually less soluble in it. The solubility of gases in plasma is about 97% of that in pure water, so that plasma under the above conditions will dissolve 97/100 × 0·48 = 0·46 c.c. oxygen. .Whole blood absorbs gases into physical solution to the extent of 92% of that of water and should, therefore, contain under these conditions 92/100 × 0·48 = 0·44 c.c. oxygen. Actually blood takes up nearly 20 c.c. oxygen. This is due to combination of the oxygen with hæmoglobin in the red cells. The oxygen held in blood in physical solution forms only a small part of the total; nearly all the oxygen is held in the corpuscles combined with hæmoglobin, and plasma only holds as much as can be absorbed by true physical solution.

The absorption of oxygen by blood can be studied by exposing it to gas mixtures with different partial pressures of oxygen and estimating the percentage oxygen saturation when equilibrium has been established. The results plotted in the form of a curve provide what is called the **oxygen dissociation curve** of the blood (Curve **A**, Fig. 22). The partial pressure of oxygen is not usually extended above 100 mm. since at this level arterial blood is practically fully saturated, or, as it is often expressed, arterial blood has an oxygen tension of about 100 mm. The oxygen tension of venous blood is about 40 mm. The partial pressure of oxygen in the alveoli is greater than 100 mm. so that when venous blood from the pulmonary artery comes in contact with alveolar air it can take up oxygen to a point approaching saturation.

The oxygen dissociation curve **A** (Fig. 22) shows that the amount of oxygen taken up increases slowly with increasing tensions up to about 30 mm., but rapidly above 30 mm. The shape of the curve is, however, considerably modified by a number of factors. The salt content of the fluid in which hæmoglobin is dissolved makes a difference. In the absence of electrolytes the curve is a rectangular hyperbola (dotted curve in Fig. 22). An increase in temperature accelerates the dissociation of oxyhæmoglobin: more oxygen is held at lower temperatures. Most interesting is the effect of pH. Acids accelerate the decomposition of oxyhæmoglobin. This can be seen by noting the effects of varying tensions of carbon dioxide on the dissociation curve. (The results are equally true for any acid producing the same change in pH). The curves **B, C, D** and **E** in Fig. 22 show that considerably less oxygen is held by blood when the CO_2 tension is high except when the oxygen tension is high, as in the lungs, and then the CO_2 has little effect. For example, in the absence of CO_2 (curve **A**) hæmoglobin is 50% saturated at an oxygen tension of 10 mm., but at a CO_2 tension of 40 mm. (the normal CO_2 tension of arterial blood) 50% oxygen saturation is only attained when the oxygen tension is 26 mm. (curve **D**). Or, at an oxygen tension of 20 mm. hæmoglobin is 82% saturated when CO_2 is absent (curve **A**), 70% saturated when the CO_2 tension is 3 mm. (curve **B**), 48% saturated when the CO_2 tension is 20 mm. (curve **C**), 37% saturated when the CO_2 tension is 40 mm. (curve **D**), and only 20% saturated when the CO_2 tension is 90 mm. (curve **E**). Since in the tissues the CO_2 tension is high and the O_2 tension low, oxyhæmoglobin will be readily dissociated and oxygen set free where it is most needed. In the lungs where the O_2 tension is over 100 mm. the hæmoglobin can be almost fully saturated even at a CO_2 tension of 40 mm.

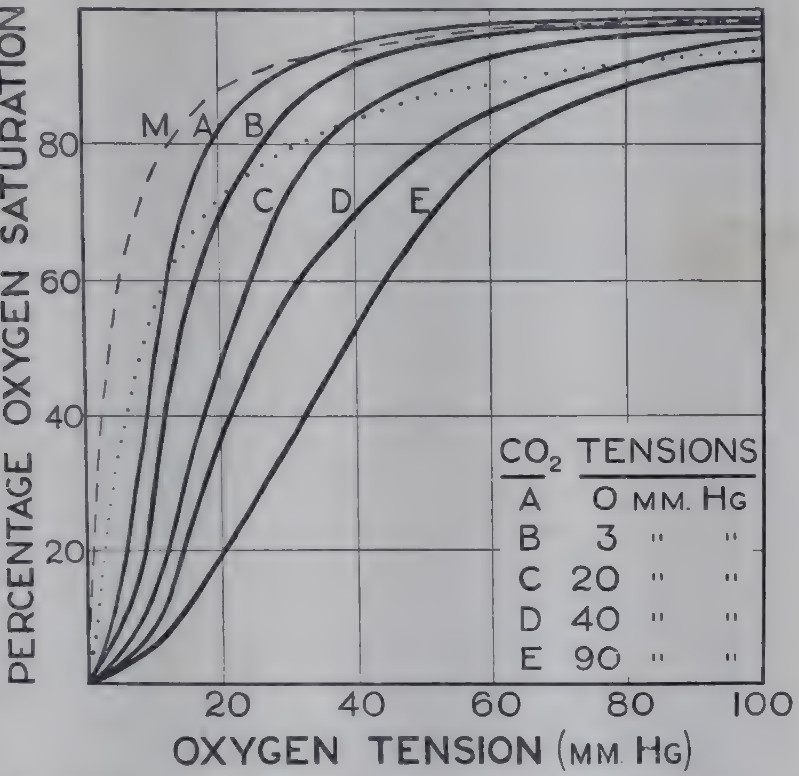


FIG. 22. Oxygen Dissociation Curves. A, B, C, D, E—Human blood exposed to different partial pressures of CO_2. Dotted curve—salt-free hæmoglobin. M—Myohæmoglobin at 40 mm. CO_2 tension. (Redrawn from Barcroft and, M, from R. Hill.)

The oxygen does not come into immediate contact with hæmoglobin either in the lungs or in the tissue cells. In both, the plasma and the tissue fluids are intermediaries. In the tissues the cell absorbs oxygen from the surrounding tissue fluid and the tissue fluid from the plasma in the capillaries. As a result of the fall in oxygen tension in the plasma, oxyhæmoglobin in the red cell dissociates giving up to the plasma oxygen which is passed on to the tissue fluid and cells.

THE CARRIAGE OF CARBON DIOXIDE BY THE BLOOD

Like oxygen, carbon dioxide is absorbed by the blood in an amount far greater than that held in physical solution. At 37° C. and 40 mm. CO_2 tension blood holds in physical solution 2·7 c.c. CO_2. Actually venous blood may hold up to 65 c.c. CO_2 per 100 c.c.; arterial blood usually contains about 50 c.c. All this CO_2 can be removed from blood by placing it *in vacuo* or by treatment with acid. It is held in the blood mainly in combination as bicarbonate, usually formulated as $NaHCO_3$ since $Na^{\cdot}$ is the chief cation or, more accurately, as $BHCO_3$ where $B^{\cdot}$ represents the sum of the cations. The $NaHCO_3$ in blood, however, does not behave exactly like a solution of $NaHCO_3$ in water. If such a solution is exposed to a vacuum only CO_2 corresponding to the equation

$$2\ NaHCO_3 \rightarrow Na_2CO_3 + CO_2 + H_2O$$

comes off. Half of the total CO_2 is still left in solution. The same applies to $NaHCO_3$ in plasma. Not all the CO_2 is evolved *in vacuo*. To estimate the total bicarbonate present in plasma, *i.e.*, the alkali reserve, it is necessary to use acid to liberate all the CO_2. But with blood all the CO_2 is evolved *in vacuo* just as if the blood had been treated with acid, *e.g.*,

$$2\ NaHCO_3 + 2\ HCl \rightarrow 2\ NaCl + 2\ CO_2 + 2\ H_2O.$$

It is the red corpuscles in the blood which are responsible for the liberation of all the CO_2. This is proved by mixing corpuscles with plasma from which as much as CO_2 as possible has already been removed by exposure to a vacuum; on evacuating again the remaining CO_2 is evolved. The substance in the corpuscles which acts like an acid in expelling CO_2 is hæmoglobin. Hæmoglobin is a weak acid and holds the base which forms $BHCO_3$ when CO_2 is added to the blood as in the tissues. This reaction can be represented

$$B.Hb + H_2CO_3 \rightarrow H.HB + BHCO_3.$$

When CO_2 escapes in the lungs or on evacuation the reaction proceeds in the opposite direction, B.Hb being reformed.

The amount of CO_2 held by the blood depends upon the oxygen tension. Blood takes up more CO_2 at low than at high oxygen tensions. This is because reduced hæmoglobin is a weaker acid than oxyhæmoglobin, *i.e.*, oxyhæmoglobin has a greater tendency to form salts. At a given pH the proportion of salt to acid will be greater for oxyhæmoglobin than for reduced, or

$$\frac{B.Hb \,.\, O_2}{H.Hb \,.\, O_2} > \frac{B.Hb}{H.Hb}$$

In the tissues the withdrawal of oxygen from the blood converts oxyhæmoglobin to reduced and thereby releases base for the neutralisation of CO_2; this enables the blood to take up more

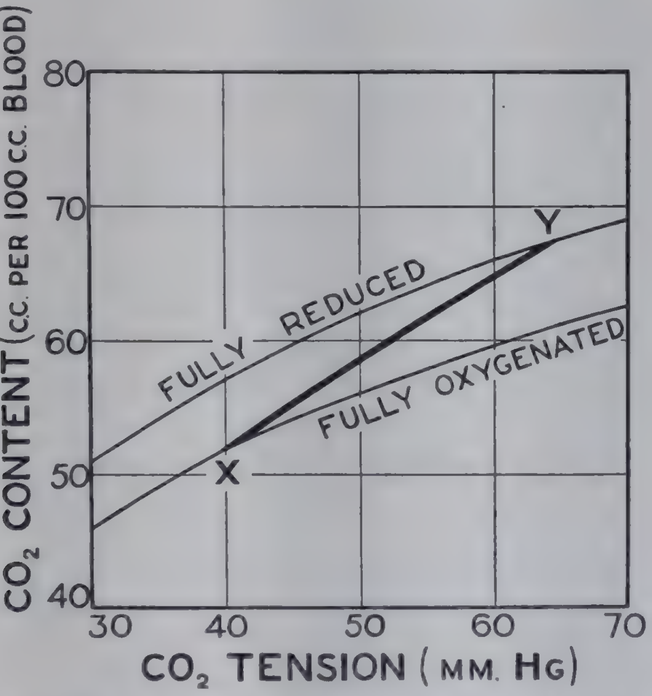


FIG. 23. Portions of the Carbon Dioxide Dissociation Curves for fully oxygenated and fully reduced human blood. The thick line XY represents the dissociation of carbon dioxide in the body. (Redrawn from Christiansen, Douglas and Haldane.)

CO_2. Conversely, in the lungs the formation of oxyhæmoglobin will facilitate the expulsion of CO_2. Fig. 23 shows portions of the CO_2 dissociation curves of fully oxygenated and fully reduced blood over the physiological range of CO_2 tension. The line **XY** represents the dissociation of CO_2 in the body.

The carriage of carbon dioxide in the blood is more complicated than that of oxygen because a considerable part of the combined CO_2 is held in the plasma ; it is not confined to the red corpuscles. Both plasma and corpuscles are well buffered and can take up considerable amounts of CO_2 because the $H^{\cdot}$ ions formed in the reaction

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^{\cdot} + HCO_3'$$

are mopped up by the proteins and phosphate of the plasma and the hæmoglobin of the corpuscles. Water exposed to CO_2 at a partial pressure of 40 mm. has a pH 4·7, but the pH of the blood under similar conditions remains 7·4. In the blood 95% of the CO_2 is combined with base as $BHCO_3$ (mainly $NaHCO_3$ in plasma) and at pH 7·4 the ratio $H_2CO_3 : BHCO_3$ is approximately 1 : 20.

When dissociation curves are constructed it is necessary to expose the blood to CO_2 for some minutes before equilibrium is established, but when blood is passing through the capillaries of the lungs or the tissues the time available for reactions to occur is very short. While most of the reactions mentioned above are extremely rapid, the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$ has been proved to be so slow that unless it were accelerated the whole chain of reactions involved in the carriage of CO_2 would be very much slower than it actually is. There is present in the red corpuscles an enzyme, **carbonic anhydrase,** which catalyses this reaction. The only other tissues where this enzyme is found in high concentration are the stomach and pancreas ; most tissues contain traces. Under appropriate conditions carbonic anhydrase accelerates the reaction in either direction. It is inhibited by traces of cyanide.

Combination of Carbon Dioxide with Hæmoglobin. The observed pH of blood is not quite in agreement with that calculated on the assumption that all the CO_2 is held as $BHCO_3$ and H_2CO_3, suggesting that some CO_2 is held in some other form, *e.g.*, combined with hæmoglobin. This is supported by experiments with blood treated with potassium cyanide to inhibit the carbonic anhydrase.

If such blood is exposed to a vacuum there is at first a more rapid evolution of a small amount of CO_2 than would be expected from $NaHCO_3$ uncatalysed by carbonic anhydrase. This is followed by a slow evolution of the bulk of the CO_2 at the expected rate. Conversely, cyanide poisoned blood takes up CO_2 at first more quickly than would be expected if the CO_2 were being fixed as bicarbonate in the absence of carbonic anhydrase. This "quick" CO_2 is combined directly with hæmoglobin, probably as a carbamino compound with the — NH_2 groups of the globin thus :

$$-NH_2 + CO_2 \rightarrow -NH.COOH$$

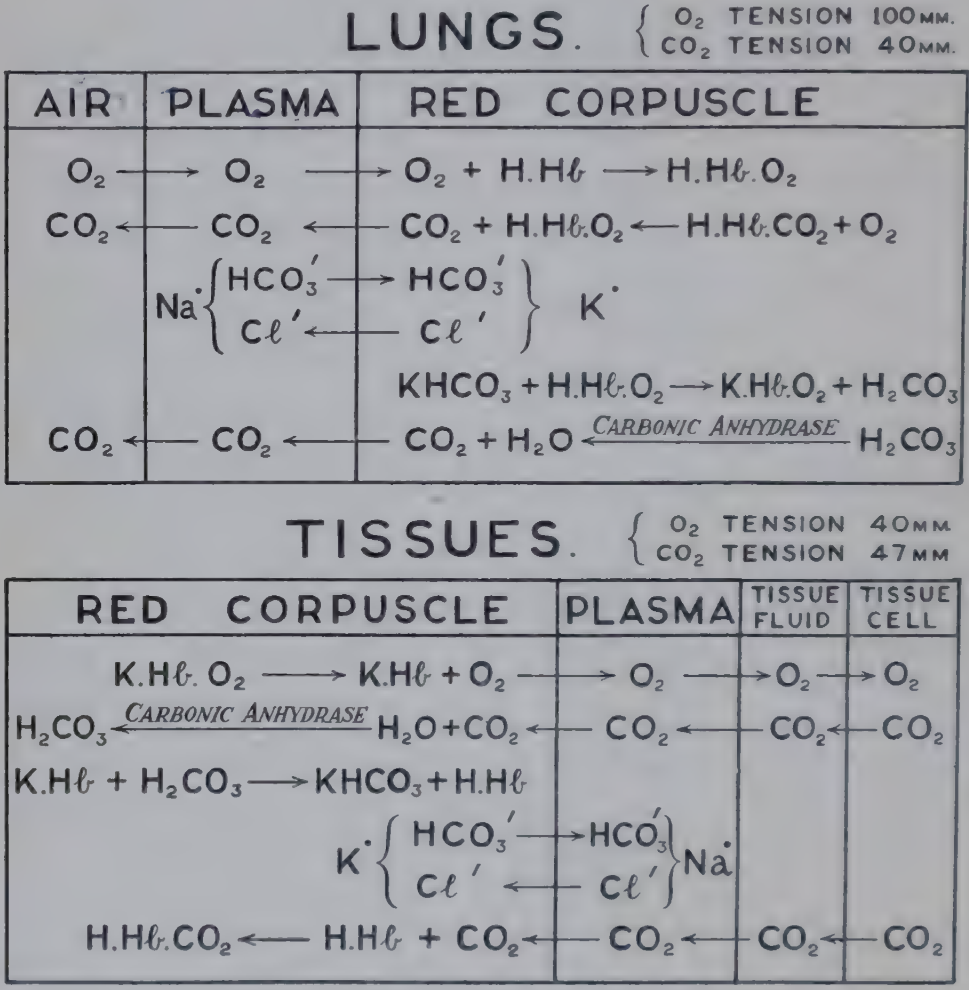
The compound formed is called **carbaminohæmoglobin** or **carbhæmoglobin** and represented $H.Hb.CO_2$. This reaction is not inhibited by cyanides. More carbamino-bound CO_2 is formed when blood is deoxygenated than when oxygenated. Oxygenation, therefore, tends to dispel carbamino-CO_2. It has been estimated that 5 to 10% of the total CO_2 in blood is carried in this way. Since, however, it is the most mobile part and represents about one-third of the difference between the CO_2 content of arterial and venous blood the carbamino-bound CO_2 forms a significant fraction of the CO_2 transported from the tissues to the lungs.

The Chloride Shift. So far we have not taken into account that hæmoglobin confers on plasma the power of fixing CO_2 in spite of the fact that the hæmoglobin is not in direct contact with the plasma but within the red corpuscle. Free diffusion of $HBCO_3$ is not possible because the red cell while readily permeable to HCO_3' ions is not permeable to cations such as $Na^{\cdot}$ or $K^{\cdot}$. Analysis of the ion content of plasma and red cells when CO_2 is absorbed or lost by blood reveals the following changes :—

	When CO_2 *is absorbed*	*When* CO_2 *is lost*
Plasma HCO_3' . .	Increases	Decreases
Plasma Cl' . .	Decreases	Increases
Red cells Cl' . .	Increases	Decreases

There is no change in plasma $Na^{\cdot}$ or red cell $K^{\cdot}$. The change in Cl' concentration when CO_2 is lost is contrary to the direction in which Cl' would be expected to diffuse since there are approximately twice as many Cl′ ions in plasma as in red cells. This change in the relative concentrations of Cl′ is referred to as the "*Chloride Shift*" or "*Hamburger Effect or Interchange*" and is explained in the following way.

Red cells are freely permeable to CO_2 and since hæmoglobin is quantitatively the most important buffer in blood and since red cells contain a high concentration of carbonic anhydrase it follows that when blood is exposed to CO_2 far more HCO_3' ions will at first be formed in the red cells than in the plasma. Since there is a higher concentration of HCO_3' ions in the red cells there will be a tendency for these easily diffusible ions to diffuse into the plasma. But, as positively charged ions such as $K^{\cdot}$ cannot accompany the HCO_3' ions since the membrane is not permeable to cations, an electrostatic field would be set up between the cells


LUNGS.
O₂ TENSION 100MM.
CO₂ TENSION 40MM.
AIR
PLASMA
RED CORPUSCLE
O₂ → O₂ → O₂ + H.Hb → H.Hb.O₂
CO₂ ← CO₂ ← CO₂ + H.Hb.O₂ ← H.Hb.CO₂ + O₂
Na· HCO₃′ → HCO₃′
Cl′ ← Cl′
K·
KHCO₃ + H.Hb.O₂ → K.Hb.O₂ + H₂CO₃
CO₂ ← CO₂ ← CO₂ + H₂O ← CARBONIC ANHYDRASE H₂CO₃
TISSUES.
O₂ TENSION 40MM
CO₂ TENSION 47MM
RED CORPUSCLE
PLASMA
TISSUE FLUID
TISSUE CELL
K.Hb.O₂ → K.Hb + O₂ → O₂ → O₂ → O₂
H₂CO₃ ← CARBONIC ANHYDRASE H₂O + CO₂ ← CO₂ ← CO₂ ← CO₂
K.Hb + H₂CO₃ → KHCO₃ + H.Hb
K· HCO₃′ → HCO₃′
Cl′ ← Cl′
Na·
H.Hb.CO₂ ← H.Hb + CO₂ ← CO₂ ← CO₂ ← CO₂

and the plasma which would oppose any further diffusion of HCO_3'. The electrical neutrality could, however, be restored by the passage of other negative ions from the plasma into the cell. The most abundant negative ion in plasma is Cl' so that there is, in effect, an interchange between the HCO_3' ions of the red cells and the Cl' ions of the plasma. This interchange enables the superior buffering power of the corpuscles to be shared with the relatively poor buffering power of the plasma. When blood loses CO_2 the migration of HCO_3' and Cl' ions proceeds in the reverse direction.

The transport of oxygen and carbon dioxide by the blood is summarised in the scheme on p. 328 showing the changes occurring in the blood in the capillaries of the lungs and in those of the tissues.

GAS EXCHANGES IN THE LUNGS AND THE TISSUES

Gas tensions in blood can be measured by equilibrating a bubble of gas of known composition with the blood and subsequently analysing the gas bubble to reveal any change in composition. The apparatus used for this purpose is called a microtonometer. If there is no change in the composition of the bubble then the tensions of the blood gases are the same as their partial pressures in the gas mixture. By this means it has been shown that the gas tensions in arterial blood, both at rest and during exercise, are practically the same as the partial pressures of the gases in alveolar air. (Actually the pressure of oxygen in alveolar air is a few mm. higher than that in arterial blood.) These pressures are 100 mm. for oxygen and 40 mm. for carbon dioxide. Typical tensions for venous blood are 40 mm. for oxygen and 47 mm. for CO_2. When venous blood reaches the lungs, therefore, there is a difference in oxygen pressure of 60 mm. favouring the passage of oxygen from the alveolar air to the blood, but only a difference of 7 mm. in CO_2 pressure tending to drive CO_2 from the blood into the alveolar air. Both pressures are more than ample for the purpose. It must be remembered that when blood reaches the lungs it is spread out in the capillaries in a very thin layer of which the total surface area is of the order of 1,000 sq. ft. This large surface of blood is only separated from the alveolar air by an extremely thin membrane so that rapid gas exchanges are possible. It has been calculated that a difference of pressure of 2 mm. would be sufficient for the oxygen required by a man at rest (250 c.c. per minute). The smaller difference in CO_2 pressure is offset by the very much greater rate at which CO_2 diffuses through wet membranes (approximately 25 times faster than oxygen). It has been shown that the partial pressure of oxygen in alveolar air must fall below 40 mm. before signs of oxygen lack are evident.

Measurement of gas tensions in the tissues is difficult but the information available suggests that oxygen tensions are of the order of 20–45 mm. and CO_2 tensions 45–55 mm. Since the values for venous blood range from 30–60 and 45–65 mm. respectively there is a pressure difference for oxygen from the blood to the tissues and for carbon dioxide from the tissues to the blood sufficient to cause the diffusion of these gases in the given directions.

MYOHÆMOGLOBIN

In muscles during activity there is an especially large utilisation of oxygen and at times the oxygen tension may fall below 20 mm. In striated muscle there is a modified form of hæmoglobin, *myohæmoglobin* (see p. 169), which assists oxygen utilisation. Myohæmoglobin differs from the hæmoglobin of blood in having an oxygen dissociation curve which is a rectangular hyperbola and which is little affected by the pH of the solution. As can be seen in curve **M**, Fig. 22, it takes up oxygen at low pressures more readily than blood hæmoglobin. For example, myohæmoglobin is 94% saturated at 40 mm. oxygen tension whereas blood under similar conditions is only 69% saturated (curve **D**), *i.e.*, myohæmoglobin could gain oxygen from venous blood. On the other hand, this greater affinity for oxygen at low pressures means that myohæmoglobin will not yield up its oxygen so readily, but this is no disadvantage in the muscle fibres since the oxidases there can work efficiently with oxygen at tensions of the order of 5 mm. The absorption spectrum of myohæmoglobin is distinct from that of blood hæmoglobin and spectroscopic measurements have shown that in tetanised muscle the oxygen utilisation is so rapid that the myohæmoglobin loses 40% of its oxygen within one second even when the oxygen supply is intact ; when the muscle is allowed to relax the myohæmoglobin takes up more oxygen again.

UTILISATION OF OXYGEN WITHIN THE CELL

The way in which the oxygen is used within the cell and the complicated chains of reactions involved have already been described in Chapter XI. It will be recalled that oxygen is not always united directly to the substance undergoing oxidation; oxidation is often a dehydrogenation and the function of the oxygen is to combine with the hydrogen to form water. For further details the reader must be referred to pages 136 to 145.

NUMERICAL DATA FOR REFERENCE

Volumes of Gases at 760 mm. Partial Pressure Dissolved by 100 Volumes of Water, Whole Blood and Plasma

	Water		*Whole Blood*		*Plasma*	
	15°	37°	15°	37°	15°	37°
Oxygen . .	3·45	2·39	3·18	2·20	3·36	2·33
Nitrogen . .	1·68	1·25	1·55	1·15	1·64	1·22
Carbon dioxide .	102·0	56·5	94·2	52·0	99·5	55·0

Typical Values for Arterial and Venous Blood

	Arterial	*Venous*
Oxygen Saturation, per cent.. . . .	96	66
Oxygen Content, c.c. per 100 c.c. . . .	19	13
Oxygen in physical solution, c.c. per 100 c.c. .	0·2	0·1
Oxygen Tension, mm. Hg.	100	40
Carbon Dioxide Content, c.c. per 100 c.c. .	50	58
Carbon Dioxide, in physical solution, c.c per 100 c.c.	2·7	3·2
Carbon Dioxide Tension, mm. Hg. . .	40	47

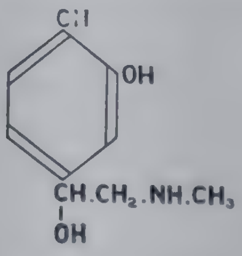
CHAPTER XXVII

THE CHEMISTRY OF THE HORMONES (1, 4)

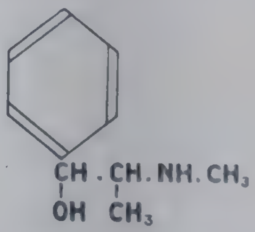
THE specific secretions of the "ductless" or "endocrine" glands which are discharged into the blood and excite some other organ or organs to specific activity are called *hormones.* It is necessary to qualify the secretion and activity as specific, in order to rule out such substances as CO_2 and urea, for these substances are formed in one organ and carried to another, where they stimulate physiological activity (respiration and diuresis). The specific activity of hormones is more the province of the physiologist than the biochemist, and here only the chemistry of the hormones will be considered. Where a hormone is concerned with definite chemical reactions, its function is discussed elsewhere; for the rest the reader must consult a text-book of Physiology. The chemistry of hormones such as **secretin** and **cholecystokinin** is practically unknown.

SUPRARENAL HORMONES

l-**Adrenaline** (*Epinephrine*), formed in the medulla of the suprarenal gland, was the first hormone to be discovered and identified chemically. The pure hormone was obtained in 1901 and subsequently synthesised by several methods.



ADRENALINE



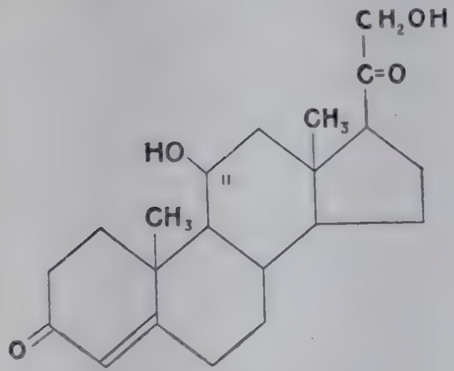
EPHEDRINE

Adrenaline is optically active, since it has an asymmetric carbon atom. The natural lævo form is thirteen times as active pharmacologically as the dextro form. More from lack of another suitable precursor than from sufficient evidence, adrenaline is said to be formed from tyrosine. A possible mechanism is given on p. 281.

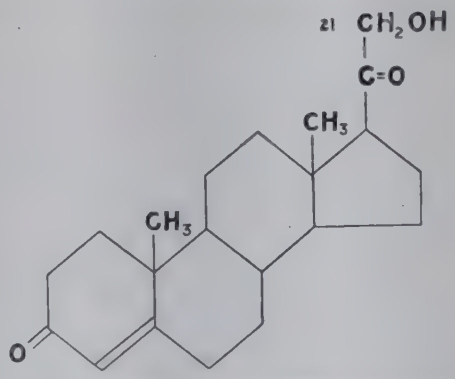
Pharmacologically, adrenaline is a powerful vasoconstrictor, increasing blood pressure by arteriolar constriction. It acts on nearly all organs with a sympathetic nerve supply, giving the same result as would stimulation of the nerves. Metabolically its most interesting action is in accelerating glycogenolysis (p. 223).

Ephedrine, a drug of plant origin having many sympathomimetic actions similar to adrenaline, is closely related to adrenaline, as shown by its formula.

Cortin (*Eucortone*). Removal of the suprarenal glands is fatal. This result is now known to be due to the lack, not of adrenaline, but of hormones produced in the cortex of the gland collectively known as *cortin*. The activity of extracts of the gland is associated with the lipide fraction from which about twenty closely related crystalline ketones and alcohols have been isolated. Several of these compounds have " cortin " activity. The most active are **corticosterone** and **desoxycorticosterone**, the latter having been synthesised before being isolated from a cortical extract.



CORTICOSTERONE



DESOXYCORTICOSTERONE

The close chemical relationship between these compounds and the sex hormones is clear if the formulæ of desoxycorticosterone and progesterone (p. 343) are compared. The only difference is that the former has an — OH substituted for — H on C_{21}. Progesterone has no " cortin " activity. Some of the compounds isolated from cortical extracts can be oxidised to ketones having properties of the male sex hormones.

Besides the crystalline compounds, an amorphous fraction possessing physiological activity has been separated from cortical extracts. Testing these compounds separately has revealed quantitative as well as qualitative differences in their action. The most striking effects of cortical extracts are on the distribution of sodium and potassium ions and on gluconeogenesis and liver glycogenesis. Desoxycorticosterone and the amorphous fraction have the greatest effect on sodium and potassium metabolism, while compounds such as corticosterone with an oxygen atom attached to carbon 11 affect primarily carbohydrate metabolism.

After extirpation of the suprarenal glands, the excretion of sodium is increased and the sodium, and hence also the chloride and bicarbonate, of the plasma is reduced. At the same time the plasma potassium rises. As a result of the lowered electrolyte content water is also lost from the plasma in order to maintain osmotic equilibrium, so that the blood becomes more concentrated and its volume reduced. Injections of cortical extracts or desoxycorticosterone prevent these changes. The condition of adrenalectomised animals is improved by administration of NaCl and aggravated by lack of it.

Hypofunction of the suprarenal cortex is observed clinically in Addison's disease, which is associated with muscular weakness, wasting, low blood pressure, and bronzing of the skin, and frequently the changes in plasma electrolytes mentioned above. Treatment with NaCl is beneficial. The symptoms and signs of experimental sodium chloride deficiency in man observed by McCance were " strongly suggestive of Addison's disease."

Administration of cortical hormones with an oxygen atom at carbon 11, *e.g.*, corticosterone, but not of desoxycorticosterone or the amorphous fraction, causes in the normal animal an increase in liver glycogen, a decrease in the rate of oxidation of glucose and an increased rate of production of glucose from protein, but not so rapid as to cause glycosuria. If the animal is partially depancreatised, the insulin available may not be sufficient to cope with the gluconeogenesis and glycosuria may ensue. Removal of the suprarenal cortex causes depletion of liver glycogen and hypoglycæmia.

Recent work has not supported the hypothesis that the cortical hormones are concerned with the phosphorylation of carbohydrate and fat.

Hyperfunction of the suprarenal cortex is associated with sexual precocity emphasising masculine characteristics. The close chemical relationship between the cortical hormones and the sex hormones has already been mentioned. (See also p. 344.)

THE HORMONE OF THE PANCREAS

The existence of **insulin,** the hormone of the pancreas essential to carbohydrate metabolism, was first suspected in 1885, when von Mehring and Minkowski found severe diabetes mellitus followed pancreatectomy in dogs ; but it was not until 1921 that an active extract of pancreas was prepared by Banting and Best. Previous failures, as pointed out elsewhere (p. 231), were mainly due to the destruction of the protein insulin by the trypsin of the pancreas. Insulin is now manufactured on a large scale. The glands are frozen immediately on removal from the animal at the slaughter-house and minced below 0° C. The insulin is obtained by extraction with weak alcohol, concentration of the extract at low temperature and precipitation of crude insulin therefrom by addition of alcohol. The crude product can be purified in several ways. Dudley's method consists in saturating the crude insulin with picric acid. Insulin picrate is separated and suspended in alcoholic hydrochloric acid, from which insulin hydrochloride separates.

Crystalline insulin was prepared by Abel in 1926 and later by Harington and Scott (1929), using a different method. These crystalline preparations are not appreciably more potent than the best commercial samples, indicating the high state of purity of the latter. Traces of zinc are apparently essential for the crystallisation of insulin. The crystals can be regarded as those of the zinc salt. The only practical source of insulin is the pancreas of slaughter-house cattle, for insulin cannot be synthesised.

Insulin is a protein with isoelectric point about pH 5·4 and molecular weight (ultracentrifuge) 35,000. The amino-acids contained include tyrosine (12%), cystine (12%), glutamic acid (30%), leucine, arginine and lysine, but not tryptophan. Insulin is fairly stable in acid, but not in alkaline solution. It is very easily inactivated by a number of reagents. The activity of insulin has been imputed to the arrangement of the sulphide and amide groupings of cystine and glutamic acid (or glutamine) and to the tyrosine groupings.

The functions of insulin are discussed under carbohydrate metabolism.

Insulin Substitutes. Many attempts at finding synthetic substitutes for insulin have been made. Many compounds which lower blood sugar (*e.g.*, synthalin) have been prepared, but their use is precluded owing to their toxicity and they cannot be regarded as insulin substitutes. See also p. 232 (protamine-insulin).

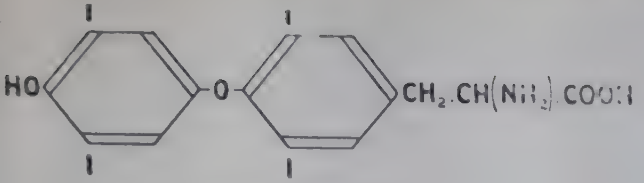
THE HORMONE OF THE PARATHYROID GLANDS (54)

Although it had long been known that removal of the parathyroid glands led to a fatal tetany, it was not until 1925 that Collip prepared an active extract of the fresh glands. The pure hormone, however, has not been isolated. This is not surprising if the supply of raw material be considered. All four parathyroid glands of an ox weigh little more than 1 g., so that co-operation with a very large abattoir is essential to get even the most meagre amount of the gland, for hormones are only present in the tissues in minute amounts. Little is known of the chemical nature of the parathyroid hormone, **parathormone,** except that it is probably associated with some form of protein. Parathormone appears to be as important in calcium metabolism as insulin is in carbohydrate metabolism. The blood calcium level is lowered by deficiency and raised by excess of the hormone. As with insulin, both effects are equally harmful. The relationship of this hormone to bone formation is described on p. 307.

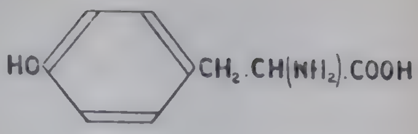
Extirpation of all the parathyroids results in tetany in a few days, which can only be relieved by injection of calcium or the hormone. Hypersecretion of the gland is associated with rarefaction of the bones as in *generalised osteitis fibrosa*, excess of the hormone causing re-solution of the calcium salts of the bones.

THYROXINE, THE HORMONE OF THE THYROID GLAND (53)

Iodine has long been known to be associated with the thyroid gland. Baumann, in 1895, observed that iodine was ten times more abundant in the thyroid gland than in any other tissue and that it was in organic combination. Several iodine-containing compounds, such as iodothyroglobulin, were isolated, but it was not until 1916 that Kendall succeeded in isolating an active substance in a state of purity. The fact that 3 tons of fresh ox gland (*i.e.*, about 50,000 oxen) were used to provide 33 g. of thyroxine indicates the laborious nature of the isolation. The constitution of the hormone was established by Harington in 1926, and confirmed by synthesis in 1927 by Harington and Barger.



THYROXINE



TYROSINE

The natural form is *l*-thyroxine, which is twice as active as *d*. Note the relationship to tyrosine. Thyroxine is an iodo derivative of hydroxyphenyltyrosine. Part of the iodine in the thyroid gland is actually present as di-iodotyrosine. The human thyroid gland (about 20–25 g.) contains about 20 mg. thyroxine. The hormone exerts a controlling influence over metabolism in general. An excess of hormone increases while a deficiency decreases the basal metabolic rate. Since injection of thyroxine does not immediately produce its physiological effects, it has been suggested that the real hormone is a peptide containing thyroxine and di-iodotyrosine, the delay in action of administered thyroxine being due to its synthesis into this peptide.

Hyposecretion of the thyroid gland in adults leads to *myxœdema*, a condition associated with thickening of subcutaneous tissue, blunting of mental activity and diminished basal metabolic rate. Administration of thyroid gland or thyroxine (orally or injected) relieves the condition. Hyposecretion in infancy leads to stunted mental and physical development, resulting in dwarfs with subnormal mental faculties usually called *cretins*. Their basal metabolism is low. The condition can be relieved by thyroxine, unless it is of long standing.

A deficiency of iodine in the diet, which sometimes occurs in districts where the water supply and the soil are poor in iodine, produces a condition known as *simple colloid goitre*, due to enlargement of the gland (but not hypersecretion of the hormone). The obvious sign is swelling of the neck. This condition is relieved by ensuring adequate supply of inorganic iodides in the food.

Hypersecretion of the thyroid is seen in *exophthalmic goitre* (*Graves' disease*). Here, there is hyperfunction of the gland and increased basal metabolic rate.

The function of the thyroid is intimately associated with a hormone of the anterior lobe of the pituitary gland, and the clinical effects described above may be primarily due to this hormone.

PITUITARY HORMONES

The pituitary body is associated with a large number of functions which have been ascribed to hormones. This is remarkable when the size of the organ (0·5 g. in man) is considered. For the purpose of preparing extracts and investigation it is only practicable to divide the gland into anterior and posterior lobes.

Anterior Lobe Hormones. At least six hormones have been allocated to the anterior lobe. They are the **Growth Hormone** associated with *acromegaly*, *gigantism* and *dwarfism*, the **Gonadotropic** and **Lactogenic Hormones** associated with sexual function, the **Thyrotropic Hormone,** absence of which leads to atrophy of the thyroid gland, the **Parathyrotropic Hormone** which influences the parathyroid gland, the **Adrenotropic Hormone,** absence of which leads to atrophy of the suprarenal cortex, the **Diabetogenic Hormone** and the **Glycotropic Factor,** influencing carbohydrate metabolism (p. 233), and the **Ketogenic Hormone** injection of which causes increased ketogenesis. None of these hormones have been isolated in a pure state. It is not even known with certainty how many substances are responsible for the effects. The methods of preparation of the extracts suggest that they are polypeptides.

Posterior Lobe Hormones. Several pharmacological actions are obtained with posterior lobe extracts. The chief of these are the *Pressor* action, due to vasoconstriction, *Oxytocic* action (contraction of plain muscle of uterus), *Anti-diuretic* action, causing delayed excretion of ingested water and possibly associated with *diabetes insipidus*, and action *antagonising that of insulin*. The last has not been extensively investigated. Chemically the active principles have been separated into two fractions, **Pitocin** (*α-hypophamine*), the oxytocic principle, and **Pitressin** (*β-hypophamine*), the pressor principle associated with the other effects. The hormonal activity of the posterior lobe would appear to be due to a protein of molecular weight about 31,000. Pitocin and pitressin, which are polypeptides of low molecular weight (about 1,000), are presumably formed from the protein in the process of separation.

Preparations of the whole posterior lobe are marketed under names such as *pituitrin*, *infundin* and *hypophysin*.

HORMONES OF THE SEX ORGANS (24)

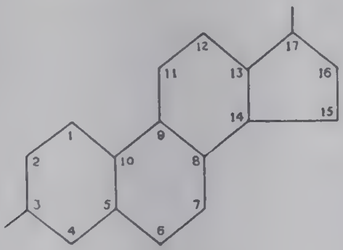
In addition to the sex-controlling hormones already mentioned, there are several hormones found in the reproductive organs themselves. These are all chemically related, and found in the lipide fraction when the organs are extracted by fat solvents. They are either ketones or alcohols. The investigation and elucidation of the structure of these hormones have been made possible by the fortunate circumstance that closely related products with similar, but lesser, activity are excreted in urine. The investigation of these relatively accessible substances from urine paved the way to the isolation and characterisation of the real hormones of the glands.

The physiological action of these hormones is beyond the scope of this volume. It is sufficient to state that they can be divided into three groups :—

Male hormones, controlling the sexual character of the male.
Œstrus-producing hormones. (Follicular hormones.)
Luteinising hormone.

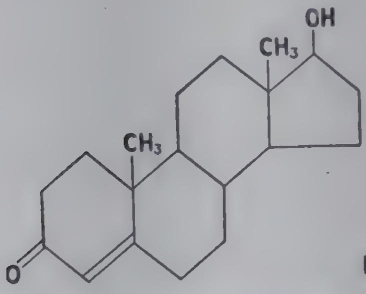
The last two together control the menstrual cycle.

All these hormones are remarkably similar in chemical structure and contain the four fused rings characteristic of cholesterol and bile acids. They have not, however, the long side chain attached to carbon 17. Oxygen attached to carbon 3 (as in cholesterol and bile acids) is a feature common to all.

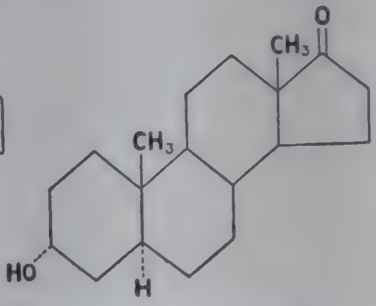


Male Hormones

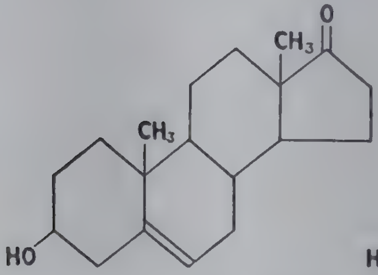
After castration, if performed at an early age, secondary sex characteristics fail to develop. Hyperfunction of the testes in boys leads to premature development of secondary sex characteristics. This is attributed to a hormone isolated from the testis and known as **testosterone.** Injection of testosterone into castrated animals produces effects comparable to those produced by the secretion of the testis. Similar results are obtained by injection of preparations from male urine which have been shown to contain not testosterone, but other less active substances called **androsterone** and **dehydroisoandrosterone,** the former being the most abundant and present to the extent of about 1 mg. per litre. The formulæ of these compounds are :—



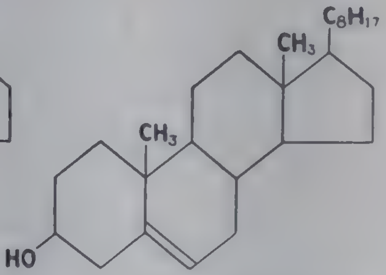
TESTOSTERONE



ANDROSTERONE



DEHYDROISOANDROSTERONE



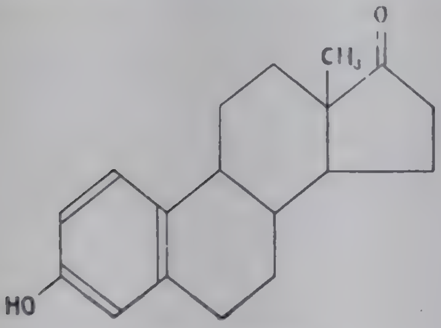
CHOLESTEROL

Note that all are dimethyl hydroxy ketones, androsterone is saturated, the other two are not. The formula of cholesterol with side chain condensed is given for comparison.

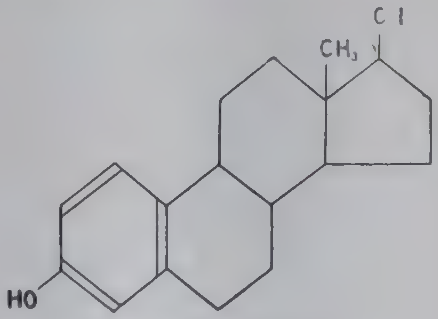
There is a tendency to regard testosterone as the hormone of the testis and the other compounds as metabolites. Several compounds with similar activity have been prepared synthetically.

Œstrogenic Hormones

These hormones, often referred to collectively as **œstrin,** produce the characteristic changes of œstrus. Like the male hormones, there are several substances having similar action and chemical structure. The best source of these substances is the urine of pregnant women or animals. The former contains about 1 mg. per litre. Pregnant mare's urine contains about 10 mg. per litre. Curiously, an even better source is the testis or urine of the stallion (but not of other male animals), which excretes more œstrin than the pregnant mare. Non-pregnant females excrete very little œstrin. The isolation of the hormone from tissues is very difficult; it is present in follicular fluid, placenta and blood. Œstrin has also been found in plants. The substance first isolated from pregnancy urines by Doisy and by Butenandt is now called **œstrone** (formerly *theelin*, *progynon*, *α-follicular hormone*, *œstrin*, etc.), and the hydrate accompanying it, isolated by Marrian, **œstriol** (formerly *theelol*, *trihydroxyœstrin*, etc.). Œstriol can be dehydrated to œstrone by heating with potassium bisulphate; 1·3 mg. œstriol per litre can be isolated from human pregnancy urine. Its activity is much weaker than that of œstrone. Reduction of œstrone gives a dihydric alcohol **œstradiol** (*dihydroxyœstrin*), which has a much greater œstrogenic activity than œstrone. The preparation of this compound in the laboratory led to its successful isolation by Doisy from sow's ovaries, one ton of which only contains about 6 mg. of œstradiol. Other active hormones (**equilin, equilenin**) have been obtained from pregnant mare's urine, but not from human urine. In structure they only differ from œstrone in that they are less saturated (see formulæ on p. 343). Œstradiol is regarded as the principal œstrogenic hormone.



ŒSTRONE

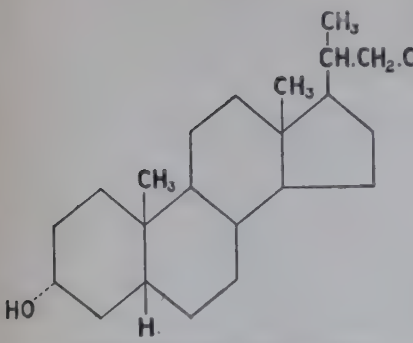


ŒSTRADIOL

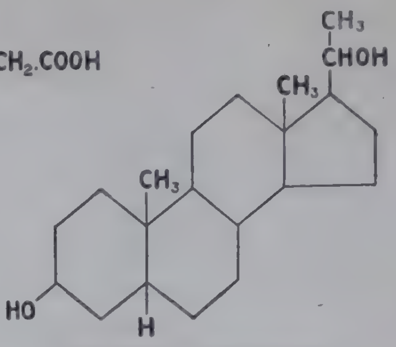
Note the close relationship between œstrone and dehydroisoandrosterone and the great difference in their physiological properties. Chemically the difference lies in a methyl group and the number and position of double bonds.

Inactive Alcohols. Accompanying the active hormones in pregnancy urine are several inactive alcohols closely related to them. Two found in human pregnancy urine are **pregnanediol** and *allo***pregnanediol**, and are recorded since they suggest a possible metabolic link between the sex hormones and cholesterol and the bile acids. The formula of lithocholic acid makes clear the close chemical relationship between the bile acids and pregnanediol.

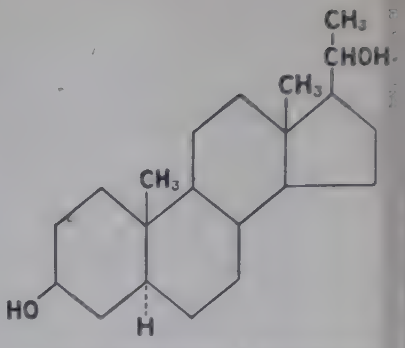
Many substances related to œstrone have been synthesised;



LITHOCHOLIC ACID



PREGNANEDIOL

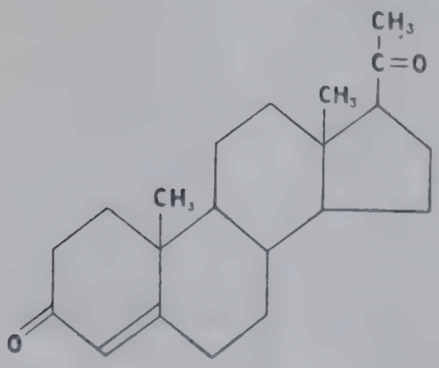


*ALLO*PREGNANEDIOL

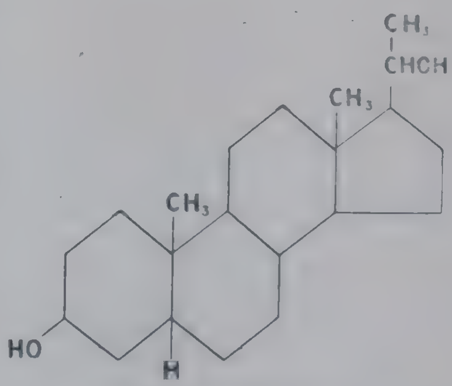
some have greater œstrogenic activity. A synthetic œstrogen which has been used clinically is *stilbœstrol* (4 , 4′-dihydroxy-α , β-diethyl stilbene). Vitamin D, which has a similar ring skeleton to œstrone, has some œstrogenic activity.

Luteinising Hormone

The preparation of the uterine mucosa for the reception of the fertilised ovum is stimulated by a secretion from the corpus luteum, which is also essential for the continuation of pregnancy. The isolation of a hormone from corpus luteum has been very difficult owing to the scarcity of material. A sow's ovaries (12 g.) only yield about 3 g. of corpora lutea ; 20 mg. of pure hormone require over 600 kg. of ovaries. In spite of these difficulties, however, sufficient pure hormone has been isolated by different workers to establish its chemical constitution. It is called **progesterone** (*luteosterone*, *progestin*)



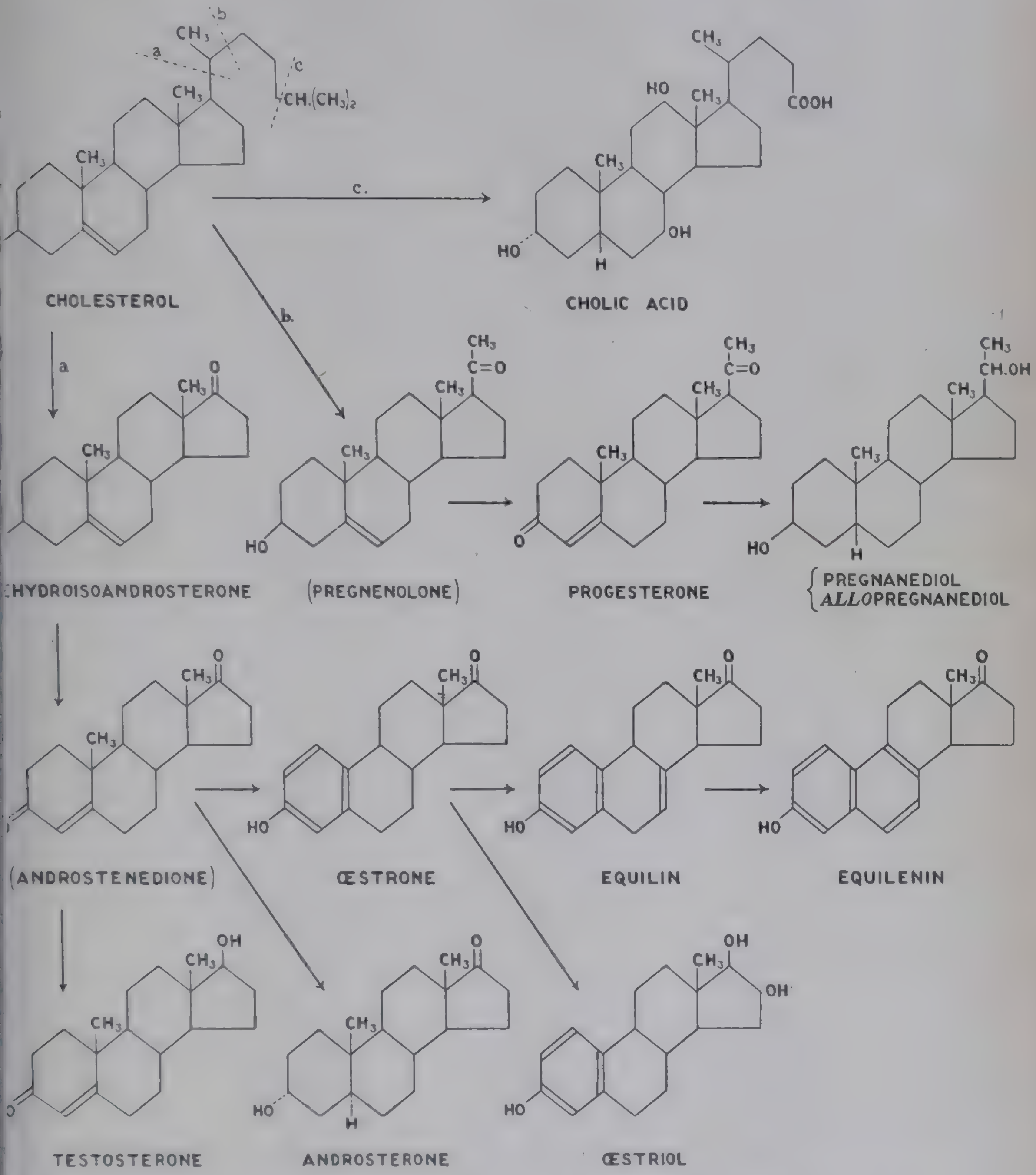
PROGESTERONE



PREGNANEDIOL

Note its close relationship to the inactive pregnanediol.

It is not yet known whether this hormone is responsible for all the activities of the corpus luteum. Unlike the other hormones in this group, progesterone is highly specific, and appears to be the only substance with this activity. By weight it is much less active than the œstrogenic hormones.


a
b
c
CH.(CH₃)₂
CHOLESTEROL
c.
CHOLIC ACID
COOH
b.
a
HYDROISOANDROSTERONE
(PREGNENOLONE)
PROGESTERONE
PREGNANEDIOL
*ALLO*PREGNANEDIOL
(ANDROSTENEDIONE)
ŒSTRONE
EQUILIN
EQUILENIN
TESTOSTERONE
ANDROSTERONE
ŒSTRIOL

On oxidation, the side chain of cholesterol could break at *a*, *b* or *c*, giving dehydroisoandrosterone, pregnenolone, or bile acids. Substances which have not been isolated are in brackets. There may be intermediate stages in many of the steps shown, *e.g.*, cholesterol to cholic acid. Modified from **Fieser, Ref. 24.)**

The Relationships of the Sex Hormones

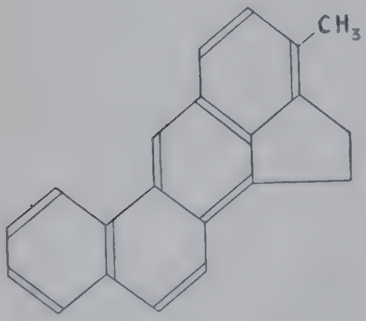
The close similarity in structure of the sex hormones and sterols and bile acids makes it tempting to assume an equally close metabolic relation, especially as cholesterol has been converted in the laboratory into most of the related compounds found naturally. The tentative scheme on p. 343 derives the sex hormones from cholesterol in a manner which is not only chemically acceptable, but in accord with known biological facts. It explains, for example, the occurrence of œstrone in male and female urine, and of the pregnanediols only in pregnancy urine. The compounds isolated from the suprarenal cortex are also closely related to the sex hormones. Corticosterone and desoxycorticosterone, for example, could conceivably be derived from pregnenolone.

Anti-Hormones

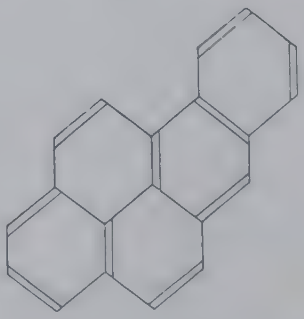
Animals become refractory to certain hormones (especially pituitary hormones) after their prolonged administration. Further, the serum of such a refractory animal may immunise other animals to the hormone under investigation. These effects are supposed to be due to the formation of *anti-hormones*, the nature of which has not yet been established.

Carcinogenic Hydrocarbons

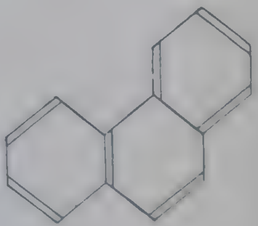
A number of complex hydrocarbons, some of which have been found in coal tar, have the property of inducing cancer in mice. Many of these have structural features common to the sterols and sex hormones. Several carcinogenic hydrocarbons, in fact, show œstrogenic activity. The suggestion has been made that carcinogenic substances might be formed in the body as a result of abnormal sterol metabolism.



METHYLCHOLANTHRENE



3, 4-BENZPYRENE



PHENANTHRENE

Methylcholanthrene, the most potent carcinogenic substance known, was actually first obtained from desoxycholic acid. **3, 4-Benzpyrene**,* which is nearly as potent carcinogenically as methylcholanthrene, has œstrogenic activity. These compounds, the sterols, the bile acids, the cortical and sex hormones all have in common the three fused six-membered rings typical of the coal tar hydrocarbon **phenanthrene.**

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* The same as the **1, 2-benzpyrene** of Cook and co-workers, who have now adopted the old numbering of pyrene which makes this compound, 3, 4-benzpyrene.

CHAPTER XXVIII

VITAMINS (55, 56, 77)

Young animals fed upon a diet of purified carbohydrate, fat, protein, inorganic salts and water in the correct proportions cease growth, and finally die. This fact was established by the experiments of Lunin (1881) and Pekelharing (1905), who both showed that the addition of small amounts of milk prevented this cessation of growth and death. Eijkmann (1906) concluded that the disease beri-beri was due to the dietary deficiency of a substance other than carbohydrate, fat, protein, salts or water, which was present in rice polishings. Hopkins (1906–1912) emphasised the importance of the "growth-promoting" substance in milk by experiments which were confirmed by Osborne and Mendel and by McCollum and Davies (1913). The latter attempted the isolation of the factor in milk and thereby showed that two factors were present. In the past, several diseases had been attributed to dietary deficiencies, since they could be cured by consuming certain foodstuffs. Hippocrates (ca. 400 B.C.) recommended ox liver and honey for night-blindness, Indians in Quebec cured an outbreak of scurvy in Cartier's men by a decoction of green leaves (1535), cod-liver oil was used in Manchester for conditions now known to be due to dietary deficiencies (ca. 1770), and barley was used to prevent beri-beri in the Japanese navy (ca. 1860).

The factors responsible for these deficiencies came to be regarded as a group to which the names accessory food factors, food hormones, vitamines (vital amines, from the mistaken idea that the substances were bases) were given; they are now called vitamins (without the "e"). The high incidence of nutritional diseases in the Great War undoubtedly provided an additional stimulus to investigation, so that to-day many vitamins and the chemical structure of several are known, although our knowledge of their functions is still elementary. A vitamin can be defined as an essential constituent of the diet, organic in nature, which is necessary for the normal functioning of the tissues, but in an amount so small as to differentiate it from ordinary foodstuffs and to suggest a controlling or catalytic function. It can be regarded as a hormone formed outside the body.

Vitamins are not universally essential throughout the animal kingdom, and different species often require different vitamins, a fact which has led to some confusion in the past and makes for the cautious interpretation of results obtained on one species only. At the present time there is evidence for the existence of at least twenty vitamins. Some of these are listed below.

Vitamin	Name	Function
A	—	Regeneration of visual purple. Prevents keratinisation of epithelial tissues.
A_2	—	
B_1	Aneurin or thiamin .	Prevents beri-beri.
B_2	Riboflavin . .	Prevents cheilosis, etc.
B_3	Williams-Waterman factor.	Essential for growth of pigeon.
B_4	Reader's factor . .	Prevents rat paralysis.
B_5	Peter's factor . .	Essential for growth of pigeon.
B_6	Adermin or pyridoxin	Prevents acrodynia in rats.
Filtrate factor II	Pantothenic acid .	Prevents dermatitis in birds.
C	Ascorbic acid . .	Prevents scurvy.
D_2	Calciferol . . .	Prevents rickets. Essential for bone development.
D_3	—	
E	Tocopherol . .	Prevents sterility.
H	Biotin or co-enzyme R	Prevents " egg-white " dermatitis in rats.
K_1	2-Methyl-3-phytyl-1, 4-naphthoquinone.	Essential for production of prothrombin.
K_2	—	
P	Citrin or eriodictyol .	Prevents fragility of capillary walls.
P-P	Nicotinic acid . .	Prevents pellagra.

The early experimental study of a vitamin consists essentially in feeding a group of young animals, generally rats, on a diet suspected to be deficient and comparing them, as far as their growth and general health are concerned, with a similar group fed on a diet known to be complete. If the animals on the suspected diet fail to grow, the absence of an essential factor is inferred; if this factor is then added to the diet, the animals should grow normally. This has given rise to the term *growth-promoting factor.* All essential constituents of a diet, including water, salts, proteins, a sufficient quantity of fats and carbohydrates, as well as vitamins, may be said to promote growth, since their absence means cessation of growth. Hence if a vitamin is described as having the function of promoting growth, it really means that there is evidence that it is essential to the animal but that its function is unknown. Later, when the function and nature of a vitamin are better known, specific methods of testing are devised. Experimental study of vitamins is fraught with difficulty since there is always a possibility of the factor under investigation being confused with one as yet unsuspected; for example, many early experiments with vitamin A were invalidated because vitamin D was then unknown (p. 354).

It is convenient to divide vitamins into fat-soluble vitamins, A, D, E and K and water-soluble vitamins, B's and C.

FAT-SOLUBLE VITAMINS

Vitamin A

Function. A number of conditions are associated with a deficiency of vitamin A, different individuals being affected in different ways. Most frequently noticed are disorders which affect the eyes. A common early manifestation of deficiency is **night-blindness** (hemeralopia, nyctalopia). A " night-blind " individual takes much longer to regenerate his visual purple when passing from bright light to dim light and may in consequence stumble. It has been suggested that when the visual purple is bleached by light, the visual yellow formed fades to colourless products and vitamin A; in the living animal vitamin A is then transformed, by combination with a protein, into visual purple (rhodopsin), thus completing a cycle. (The retina of the hog is remarkably rich in vitamin A.) Night-blindness has long been known, as well as its rapid cure by taking liver oils. Outbreaks have been observed in prison camps, badly fed communities (*e.g.*, Newfoundland and Labrador coasts), and during religious fasts, but they are not common in western Europe. Measurement of the dark adaptation has recently been applied as a test for slight hypovitaminosis A in man. An individual with a suboptimal amount of vitamin A shows great improvement in dark adaptation after taking a dose of vitamin A

A common indication of deficiency, once thought typical, is **xerophthalmia** (dry eye), caused by keratinisation of the cornea of the eye which may lead to permanent damage if not treated early. If vitamin A is taken, recovery is usually rapid. This condition also is not common in western Europe although sudden outbreaks have been produced by reduction of the amount of vitamin A in the diet. During the war of 1914–1918, Denmark exported large quantities of butter and used margarine and skim milk for home consumption ; this caused a high incidence of xerophthalmia in young children, but the number of cases fell considerably when the export of butter was restricted. Xerophthalmia is more common in undernourished communities in the Orient.

It has frequently been noticed that xerophthalmia is associated with a decreased resistance to bacterial infection. The Danish children were remarkably susceptible to bronchopneumonia ; experimental animals show an increased susceptibility to infections of the respiratory tract. Vitamin A was in consequence termed the anti-infective vitamin. Careful experiments, however, have failed to reveal any direct connection between the vitamin and infection ; administration of the vitamin does not improve the infective condition. There is no conclusive evidence that vitamin A prevents the common cold. It would seem that the increased susceptibility to infection is that of a damaged tissue rather than a specific effect due to absence of the vitamin, for many epithelial tissues, *e.g.*, the respiratory tract, and, in animals, the alimentary and genito-urinary tracts, may keratinise if vitamin A is lacking. Evidence is steadily accumulating to show that a deficiency of vitamin A coupled with a high intake of calcium favours the formation of calcium phosphate stones in the kidney and bladder. Such stones have been induced experimentally in animals on low vitamin A high calcium diets. A liberal intake of milk almost certainly prevents the formation of such stones.

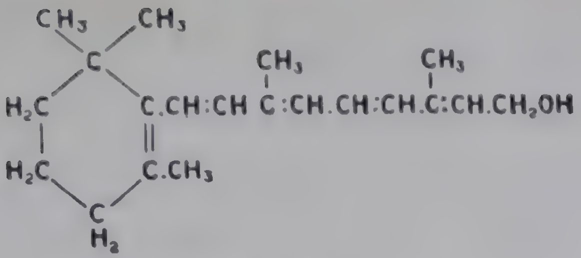
Periodontal diseases such as *gingivitis* or *pyorrhœa* have been ascribed to vitamin A deficiency. A dermatitis known as *phrynoderma* or "*toad skin*" has been attributed to lack of vitamin A. Moore has noted subnormal amounts of vitamin A in human livers in many diseases. Mellanby has produced deafness in dogs by feeding them on vitamin A-deficient diets, but it is not yet known whether this has human application.

In properly fed individuals the effects of vitamin A deficiency are not observed until the liver stores are exhausted (several weeks). Adults are less liable to be affected than are children since the former have a greater store of vitamin. Undernourished individuals respond quickly to a vitamin A-deficient diet.

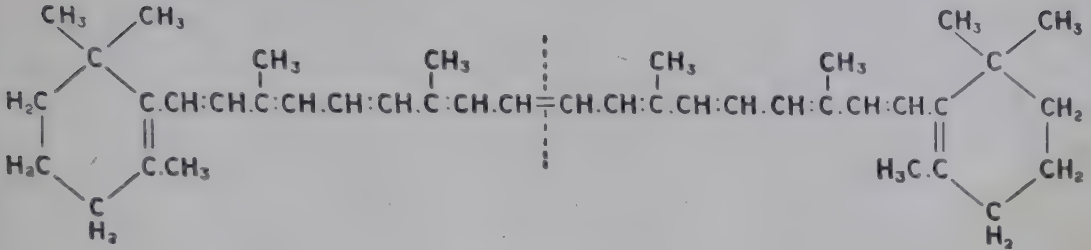
Chemical Nature. By fractionation of the unsaponifiable fractions of certain fish liver oils vitamin A has been obtained as pale yellow needles (m.p. 7·5–8·0° C.). Amongst the characteristic properties are an absorption band in the ultra-violet spectrum at 328 mμ, behaviour as an unsaturated alcohol which is stable in the absence of air, and the formation of a blue colour when treated with antimony trichloride.

Steenbock, studying the vitamin A of plants, traced a correlation between their vitamin activity and content of carotene-like pigments, but when it was found that pale-coloured animal oils were highly active and that pure carotene did not support the growth of rats deprived of vitamin A, the idea of a relation between carotene and vitamin A was abandoned. (Carotene does not show absorption at 328 mμ and gives a different colour with antimony trichloride.) Later, when crude vitamin A was shown to give the same decomposition products as carotene, the pigment was re-examined with the result that it was found to have a very high vitamin activity. The error in the earlier test of carotene was due to the diet of the rats being deficient in the then unknown vitamin D ; the rats failed to grow, not because of lack of vitamin A but through lack of vitamin D. A biological relation between the orange carotene and the nearly colourless vitamin A was established when it was shown that carotene, when fed to the rat, pig and other animals, was deposited in the tissues, especially the liver, as the nearly colourless vitamin A. An oil 2,600 times as active as cod-liver oil has been obtained in this way. The mechanism and site of this conversion are unknown. It is probable that it is not the same in different species. There is some evidence that vitamin E is concerned in this conversion.

The structure of the carotenes is given on p. 120. β-Carotene is more active than the α or γ isomer. Lycopene and lutein are not converted into vitamin A which contains the β-ionone ring (Ring I on p. 120). β-Carotene contains two β-ionone rings, α- and γ-carotenes only one. Of several artificial carotenes only those with a β-ionone ring show vitamin A activity. The recent synthesis of vitamin A supports Karrer's formula



VITAMIN A



β-CAROTENE

which represents half the β-carotene molecule with H_2O added at the point of rupture. β-Carotene would presumably form two molecules of vitamin A, α- and γ-carotenes only one.

Examination of the livers of fresh water fish has revealed the existence of another vitamin A, **vitamin A_2**, which is similar to vitamin A both biologically and chemically. Its structure is probably that of A with an additional $-CH=CH-$ inserted at the end of the side chain next to the $-CH_2OH$.

Sources. It will be clear from the above that we shall have two effective sources of vitamin A, foods which contain the vitamin and those which contain carotenes. So far, the existence of vitamin A has not been proved in plants, so that vitamin A will only be obtained from animal foods. The carotenes are present in most plants. Animal tissues contain varying amounts, for ingested carotene is only slowly and not necessarily completely converted into vitamin A. Carotenoid pigments have always been regarded as almost inevitable concomitants of animal fats.

Whilst most fat-containing tissues contain vitamin A, the liver is the main store, and therefore the richest source. The liver oils of mammals and certain fish (*e.g.*, halibut *) may be several hundred times more potent than cod-liver oil, which is, however, the most abundant commercial source. The activity of a given

* Some halibut liver oils contain as much as 3% of vitamin A.

tissue will depend upon the previous diet and age of the animals. Those plentifully supplied with carotene will provide vitamin-rich livers. The activity of cod-liver oils * varies greatly, those of young fish having only about a quarter the activity of full-grown; this probably accounts for the big variations in the activity of commercial specimens of the oil.

The variations in summer and winter milk and butter are due to the difference in carotene content of fresh grass and hay and oil-cake. Carotene is destroyed when hay is sun dried in the ordinary way, but preserved if the grass is rapidly dried artificially. On a given pasture some breeds of cows (*e.g.*, Shorthorn) convert more carotene into vitamin than do others (*e.g.*, Guernsey) which secrete a yellower milk, although the sum of vitamin + carotene is roughly equal.

In plants the distribution of carotene corresponds roughly to that of chlorophyll, so that carotene is found most abundantly in the greener (*i.e.*, outer) leaves. In addition, some plants store carotene; of these, carrots, whose colour is largely due to carotene, are the only rich source. Of cereals, yellow maize is the only significant source. Most vegetable oils † and yeast show no vitamin A activity.

Vitamin A and carotene are only destroyed in cooking or preserving processes involving exposure to air at high temperatures, as in frying, or for long periods, as in sun-drying.

Vitamin D

Function. Vitamin D protects against rickets, and is therefore called the antirachitic vitamin. How it does so is still disputed. **Rickets** is a disease, formerly very prevalent among children, associated with malformation of the bones, due to deficient deposition of calcium phosphate. That such bones are unable to stand the normal strain is evidenced by the appearance of bow-legs, knock-knees, pigeon-chest and frontal bossing of the skull. The equivalent diseases in adolescents (**late rickets**) and adults (**osteomalacia**) are probably brought about by the same causes. The differences in the clinical picture, *e.g.*, the tendency of the bones to fracture rather than bend, are chiefly due to the state of the bones at the onset of the disease. There are probably several factors concerned, including : (*a*) deficiency of calcium or phosphorus ; (*b*) unbalanced calcium and phosphorus ; (*c*) deficiency of vitamin D ; (*d*) lack of sunlight ; the condition is accompanied by an increased blood phosphatase (see Chapter XXIII). In the commonest form, *low-phosphorus rickets*, the plasma inorganic phosphate is low and the plasma calcium is often normal ; in *low-calcium rickets*, in which the serum phosphate is usually normal, but calcium low, tetany (*cf.* p. 307) is frequently observed. The present tendency is to regard rickets as essentially a blood disease, the condition of the bones being a consequence of the disturbed calcium and phosphorus balance of the blood. Rachitic ossifying cartilage contains adequate phosphatase and readily calcifies *in vitro* if immersed in a solution of salt content equivalent to normal serum. In rickets the plasma phosphorus or calcium (or both) are so reduced that the bone phosphatase cannot raise the inorganic phosphate sufficiently to exceed the solubility product $[Ca^{\cdot\cdot}]^3 \times [PO_4{}''']^2$, and cause deposition of bone. On administration of vitamin D normal calcification ensues, provided vitamin D is the only deficiency,* but the deformities are not corrected. Rickets is frequently complicated by a dietary deficiency of calcium and phosphorus, but occurs on a normal intake of these elements if vitamin D is lacking.

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* The origin of the vitamin A is presumed to be ultimately carotene formed in marine diatoms by photosynthesis. It is probable that intermediate products devoid of vitamin activity are formed in smaller marine forms before being converted to vitamin A in the cod.

† Palm oil is a notable exception in containing a high percentage of carotene.

In some way vitamin D controls the amount of calcium and phosphorus which can be utilised. The supposed effect of vitamin D in assisting in their absorption from the intestine may be a prevention of re-excretion into the gut ; it is, perhaps, significant that in rachitic rats (but not in children) the reaction of fæces is less acid than normally, or may even be alkaline. By very excessive dosage of vitamin D it is possible greatly to raise the calcium and phosphate level of the blood (and diminish the fæcal excretion) and cause calcification in regions which normally do not calcify,† *e.g.*, in the aorta and kidney. Since excessive dosage with parathyroid extracts raises the blood calcium and phosphate levels with somewhat similar results, it has been suggested that vitamin D acts by stimulating the parathyroids; as yet there is no convincing proof of this. For the moment it may be concluded that vitamin D controls the retention of calcium, whereas the parathyroids regulate blood calcium by withdrawing it from the bones. The reciprocal relation between calcium and phosphorus has already been remarked upon (p. 301); in the present state of our knowledge of the metabolism of these elements it is very difficult to differentiate between cause and effect and assign to one element the primary *rôle*.

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* Calcium might be lost to the body by excretion as calcium soaps, due to failure of fat absorption, as in cœliac rickets. This condition would not be cured by vitamin D.

† There is no risk of these effects of hypervitaminosis D occurring from the consumption of ordinary foodstuffs.

Chemical Nature. In 1919 Mellanby provided convincing evidence that rickets was due to deficiency of a fat-soluble dietary constituent which was supposed to be vitamin A, since D was unknown at the time. Rickets could be cured by cod-liver oil. Simultaneously other workers proved equally clearly that rickets could be cured by exposing the children or animals to sunlight, or more specifically, ultra-violet light of wavelength about 300 $m\mu$. In 1922 McCollum showed that, while cod-liver oil lost its vitamin A by oxidation, it remained active in curing rickets, thus proving the existence of another fat-soluble vitamin (D) in the oil. The connection between vitamin D and ultra-violet light was revealed in 1924 by Steenbock and Hess, showing that certain foodstuffs devoid of vitamin D could be rendered antirachitic by exposure to ultra-violet light. The substance activated by ultra-violet light was finally traced to an impurity present in small amounts in the cholesterol fraction of the foodstuffs. Of the known sterols, irradiated (*i.e.*, exposed to ultra-violet light) **ergosterol** provided the most potent antirachitic substance, which was therefore regarded as crude vitamin D. From it a pure white crystalline substance with four times the original activity was finally isolated. It is called **calciferol** in this country and vitamin D_2 in Germany.*

Calciferol is isomeric with ergosterol and there are two inactive isomeric sterols (lumisterol and tachysterol) formed as intermediate products. Over-irradiation gives other inactive sterols, some of which are toxic. The formulæ of ergosterol and its active irradiation product are:—

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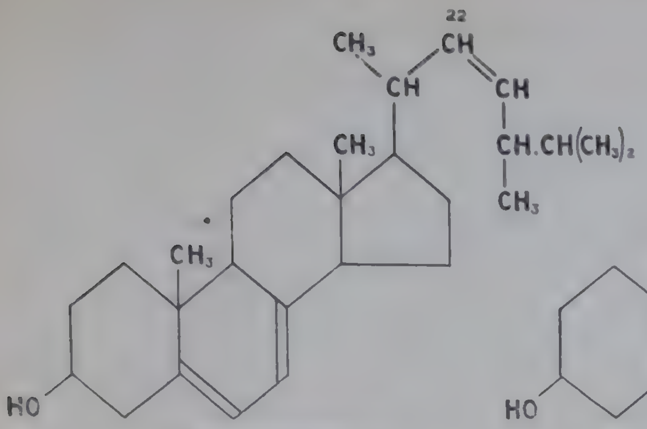
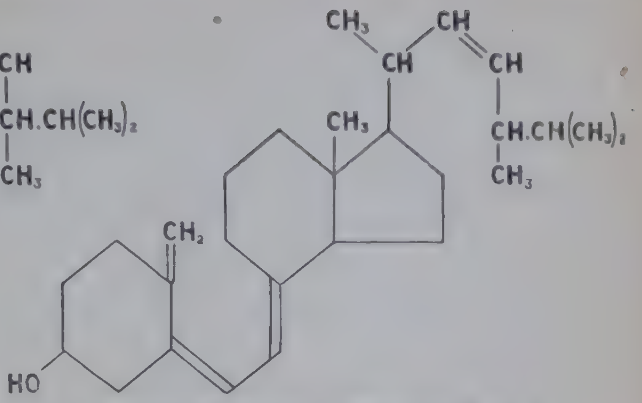
* At first, products with twice the activity of irradiated ergosterol were obtained and called calciferol and vitamin D in the respective countries. On it being shown that they were mixtures of an active and an inactive sterol, the name calciferol was retained for the active product in this country, but changed to D_2 in Germany.


22
CH3
CH
CH
CH
CH.CH(CH3)2
CH3
CH3
CH3
HO

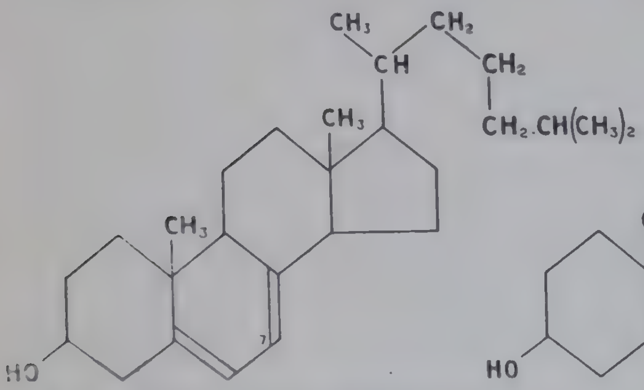
ERGOSTEROL


CH3
CH
CH
CH
CH.CH(CH3)2
CH3
CH3
CH2
HO

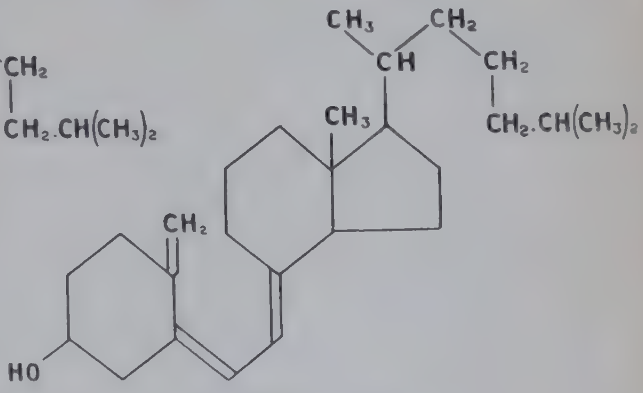
CALCIFEROL (VITAMIN D_2)

Note that ergosterol differs from cholesterol (p. 85) in having an extra CH_3 and two extra double bonds. The formation of calciferol involves rupture of the second ring.

Antirachitic substances with similar activity have also been obtained by irradiation of **22-dihydroergosterol** (ergosterol with a saturated side chain) and **7-dehydrocholesterol** (cholesterol with hydrogen lost from and, therefore, a double bond between carbons 7 and 8; *i.e.*, ergosterol with the side chain of cholesterol). Note the difference between dihydro- and dehydro-.


CH3
CH2
CH
CH2
CH3
CH2.CH(CH3)2
CH3
7
HO

7-DEHYDROCHOLESTEROL


CH3
CH2
CH
CH2
CH3
CH2.CH(CH3)2
CH2
HO

VITAMIN D_3

The active product from 7-dehydrocholesterol has been isolated in the pure state by Windaus and called vitamin D_3.

If samples of calciferol and cod-liver oil which are quantitatively equal when assayed upon rats are tested on chicks, calciferol is less potent, showing that calciferol is not identical with the natural vitamin in cod-liver oil. Both 7-dehydrocholesterol and vitamin D_3 have recently been isolated from the liver oil of tunny fish, so that D_3 is identical with the natural D of tunny-liver oil, but it is not certain that D_3 is the vitamin of all fish oils. Some workers maintain that there are at least six D vitamins.

The D vitamins are more stable than vitamin A and withstand ordinary cooking and preserving processes; destruction by heat when exposed to air, as in frying, is slower than with vitamin A.

Sources. The richest sources of vitamin D are the liver oils of fish; mammalian liver oils, although rich in vitamin A, contain very little vitamin D, even after a diet rich in the vitamin. Egg-yolk is a good source if the bird has had an adequate supply of vitamin D or exposure to sun- or ultra-violet light. Other foodstuffs, including milk, are relatively poor sources of vitamin D, although the amount in milk can be increased by providing additional vitamin D in the cow's diet. Green vegetables by the time they reach the market are practically devoid of vitamin D.

The cheapest source of vitamin D is sunlight, which forms D_3 from 7-dehydrocholesterol in the skin; it is human experience that the need for dietary vitamin D is inversely proportional to the incidence of sunlight on the skin. In the far north Eskimos depend entirely on diet (fish oils), whereas natives in the tropics need little vitamin D in food.

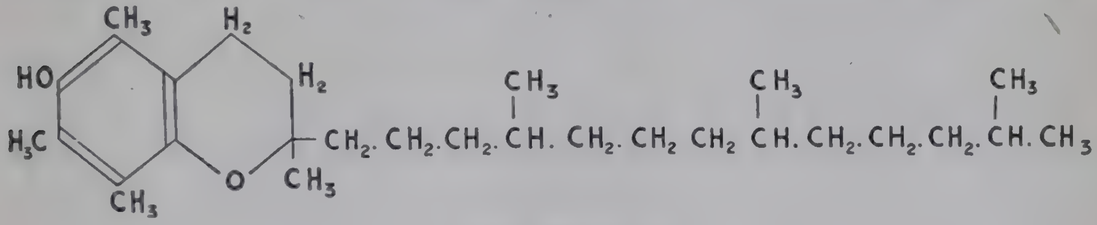
Vitamin E

Function. In early vitamin experiments it was frequently noticed that rats on a supposedly complete artificial diet failed to breed, or rear young if born. This was ultimately traced by Evans to a factor present in vegetable fats and called vitamin E. In the absence of this vitamin young rats grow normally except for a diminution of reproductive power. In the male, damage to the testis may result in permanent sterility; in the female, conception can occur, but the embryo dies within two weeks and is resorbed. If a litter is born alive in spite of some deficiency of vitamin E, lactation may be defective. These effects are prevented if the animals are supplied with vitamin E. The difficulty of correlating these conditions has led to the suggestion that more than one factor is present; it must, however, be remembered that reproductive function is associated with many hormones which may work in conjunction with vitamin E. An excessive intake of the vitamin does not increase fertility above normal. The vitamin is stored in the tissues, especially in muscle and body fats, and effects of a dietary deficiency are long delayed. The successful treatment of a few cases of sterility in women and cows by vitamin E concentrates has been reported. Goats and sheep are not affected by vitamin E deficient rations.

Guinea-pigs, rabbits and rats on diets deficient in vitamin E develop a primary muscular dystrophy resembling the progressive muscular dystrophy of man. The condition responds rapidly to treatment with vitamin E concentrates. The treatment of cases of human muscular dystrophy with the vitamin has met with some success particularly in children, although the recovery is very much slower than with animals.

There is evidence that vitamin E is in some way concerned with the conversion of carotene into vitamin A.

Chemical Nature. Three unsaturated alcohols, called, α-, β- and γ-**tocopherol**, have been isolated from the unsaponifiable fraction of wheat-germ oil. The structure of α-tocopherol is given below. β- and γ-tocopherol differ from α-tocopherol in having only two methyl groups in the aromatic nucleus. All three compounds are active, but the amounts isolated represent only a fraction of the activity of the wheat-germ oil.



α-TOCOPHEROL

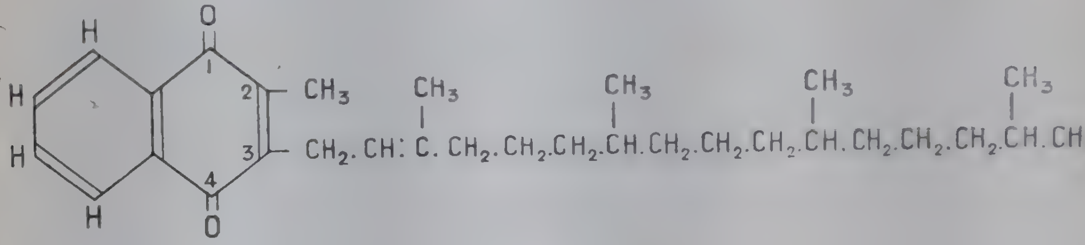
Sources. The best source of vitamin E is wheat-germ oil. The fats of many cereal and other plant seeds (but not all) are rich sources, as are the green leaves of spinach, watercress, lettuce and alfalfa (lucerne), and egg-yolk. The vitamin is only present in small amounts in milk, animal tissues and cod-liver oil.

Vitamin K

This fat-soluble vitamin is essential for the chicken, duck, goose and pigeon and possibly for mammals. In its absence the clotting time of blood is delayed and this fact has been applied clinically.

Reduction in clotting time has been observed when preparations of vitamin K are given to patients suffering from obstructive jaundice. (Delayed clotting time is often noted in this condition.) Vitamin K appears to be necessary for the production of prothrombin, and has been used with success in the treatment of hypoprothrombinæmia in the new born. Human deficiency of the vitamin is unlikely except on very restricted diets. The absorption of the vitamin is assisted by bile salts which should be given simultaneously if the vitamin is administered to patients with biliary obstruction.

Two K vitamins have been found naturally. K_1, obtained from alfalfa, is 2-methyl-3-phytyl-1, 4-naphthoquinone. K_2, obtained from putrid fish meal, is also a 2, 3-disubstituted 1, 4-naphthoquinone. 2-Methyl-1, 4- naphthoquinone is as effective as either of the natural vitamins.


H
O
H
H
H
O
1
2
3
4
CH3
CH3
CH3
CH3
CH3
CH2. CH: C. CH2.CH2.CH2.CH.CH2.CH2.CH2.CH.CH2.CH2.CH2.CH.CH3

Vitamin K_1

Vitamin K_1 is found in the green leaves of alfalfa, spinach, carrot tops, kale, etc. Vitamin K_2 has been shown to be produced by bacteria which are frequently present in the human alimentary canal, so that the vitamin may be produced in the intestine even if it is not supplied in the diet.

WATER-SOLUBLE VITAMINS

About ten years ago two water-soluble vitamins, B and C, were recognised. To-day, while C retains its individuality, B has been differentiated into several factors. The term B_1 is retained for the original factor protecting against beri-beri. Four factors have been isolated and chemically identified from the original vitamin B_2 or "vitamin B_2 complex." They are riboflavin, nicotinic acid, pyridoxin and pantothenic acid. The existence of several other B factors, including folic acid and biotin, has been established. Some of these are listed on p. 347. Vitamin

B_3 is possibly identical with pantothenic acid, and B_6 with pyridoxin. Vitamin B_1, riboflavin, nicotinic acid, pyridoxin and ascorbic acid all play a part in oxidation-reduction systems. (See pp. 129, 142).

Rats can synthesise most of the B vitamins by the action of bacteria upon food residues in the cæcum. The extent of the synthesis is influenced by the diet. Experiments on man suggest that vitamin B_1, riboflavin and nicotinic acid can be formed in the bowel although there is such very great individual variation in the amounts synthesised that one man may form none whilst another may produce his whole requirement.

Vitamin B_1 (Aneurin, Thiamin)

Function. Since beri-beri is accompanied by loss of appetite, the clinical picture is often confused by superimposed deficiencies. Usually a general numbness leading to paralysis of the limbs and incapacity for physical work are observed. The condition, unless complicated by other deficiencies, is rapidly relieved by vitamin B_1 (F in America). In experimental animals with a single deficiency of B_1, there is loss of appetite, followed by multiple peripheral neuritis, polyneuritis (hence the description of B_1 as the antineuritic vitamin or aneurin). In pigeons there is a characteristic head retraction (opisthotonus) as well as paralysis of the legs and wings, leaving the bird helpless. The recovery on giving B_1 is remarkably rapid. The neuritic condition is due to a disorder of the central nervous system. Peters has shown that in B_1 deficiency lactic acid accumulates in the brain, but disappears on addition of the vitamin ; this can be shown both *in vivo* and *in vitro*. The brain of a B_1 deficient pigeon takes up less oxygen than a normal brain when lactic acid or pyruvic acid is added. Vitamin B_1 is probably a co-enzyme for the oxidation of pyruvic acid,* which is the first stage in the oxidation of lactic acid in the brain. The blood of a B_1 avitaminous pigeon or rat contains an abnormal amount of pyruvic acid which disappears upon the cure of the animal. Platt has recently observed that in cases of human beri-beri the blood pyruvic acid is increased and that the normal level is restored after intravenous injection of 5 mg. of pure vitamin B_1.

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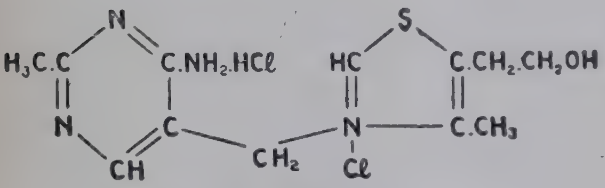
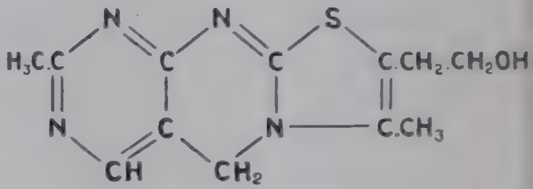
* Vitamin B_1 combined with pyrophosphate (co-carboxylase) is an important catalyst for one stage in the formation of alcohol from glucose by yeast. The reaction catalysed by co-carboxylase is $CH_3 . CO . COOH \longrightarrow CH_3 . CHO + CO_2$. It has been shown that vitamin B_1 pyrophosphate is present in brain tissue. For formula, see p. 130.

Another conspicuous sign of vitamin B_1 deficiency in animals is hypertrophy of the heart ; administration of B_1 causes a rapid return to the normal size. In rats there is a marked **bradycardia** (slowing of the heart) with return to the normal heart rate on injection of an appropriate amount of B_1. This forms the basis of a method of assay.

Chemical Nature. The absence of a really potent source of vitamin B_1 (about ½ g. can be isolated from 2 tons of yeast) made the isolation of the pure vitamin very difficult. It was finally isolated by several workers in 1933 and its constitution established and confirmed by synthesis in 1936.



VITAMIN B_1 (ANEURIN) HYDROCHLORIDE



THIOCHROME

Note the presence of the pyrimidine ring and sulphur. The pure vitamin has been called **aneurin** and **thiamin.** On oxidation it yields a yellow dye with a blue fluorescence called *thiochrome.* Vitamin B_1 is fairly stable to cooking and modern canning processes, although it may be lost in boiling owing to its solubility in water. It is destroyed by boiling in an alkaline medium. There is no loss in baking bread provided baking powder is not used.

Sources. The chief sources of vitamin B_1 are cereals, pulses and nuts. Eggs (yolks) contain a fair amount. Green vegetables and animal tissues contain the vitamin, but are probably of only minor dietary importance. Yeast and yeast extracts are probably the richest sources, although the vitamin content of different yeasts varies greatly.

Dietetically, bread and rice are of most interest. Vitamin B_1 is almost confined to the embryo of the cereal grain. Modern milling processes yielding white flour and white (polished) rice remove this embryo along with the husk and consequently nearly all of the vitamin. Beri-beri was rare before the end of the nineteenth century, except in definitely undernourished communities, because cereals and rice were not milled to remove the embryos. The chief reason for the modern milling processes was the necessity of providing a grain which would keep and transport better than wholemeal flour, in which the embryo fat was liable to rancidity, and brown rice which was liable to become infested with weevils on long voyages. The more pleasing appearance and better selling power of white bread undoubtedly encouraged the process, so that at the beginning of this century the use of white bread was almost general in towns. Since rice forms the chief article of diet in the Far East, beri-beri became very common about the end of the last century. The experiments of Eijkmann and others made it quite clear that return of the rice polishings to the diet prevented the disease in prisoners in Java and the Philippine Islands. The disease was stamped out in the U.S. Army Corps in the latter by the inclusion of unpolished rice and beans in the diet. Before 1914 more than 800 children in Manilla died of beri-beri annually ; since 1918 this mortality has been reduced to zero. Infantile beri-beri in breast-fed infants is common in the Far East in undernourished communities. The milk of mothers on a deficient diet does not contain sufficient vitamin B_1 for the child. The amount of this vitamin in cow's milk is small.

In Europe and U.S.A., frank beri-beri is rare in spite of the large part white bread plays in our diet. This has been ascribed to the vitamin being obtained from other sources in our mixed diet, although some maintain that many digestive disturbances are really a sign of a suboptimal intake of vitamin. There is ample evidence that abnormal conditions produce beri-beri ; an outbreak of beri-beri in a New Jersey prison was checked by changing from white bread to brown ; British troops in the Near East during the Great War suffered from beri-beri on a diet consisting largely of white flour and tinned foods, whereas Indian troops on wholemeal flour were free. A Danish whaling expedition provides the record of 51 cases of the disease on one ship on which no wholemeal bread was consumed, with no cases on five ships using wholemeal bread.

Riboflavin (Lactoflavin, Vitamin B_2)

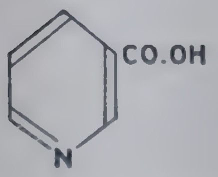
Riboflavin, or vitamin B_2, is required by man, dogs, rats and chickens. Cases of human ariboflavinosis responding to administration of riboflavin, have been observed in the southern states of

U.S.A. and Singapore. Physical signs show cheilosis, dermatitis, lesions of the eyes, mouth and tongue, and weakness of the legs. Riboflavin in the cell is associated with protein and phosphoric acid and can act as an oxidising enzyme (see p. 142) but it is not known whether this function is associated with the physiological effects of a deficiency.

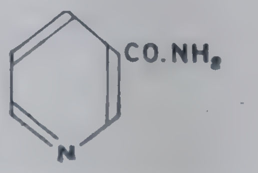
The formula of riboflavin, confirmed by synthesis, is given on p. 119. It was first isolated from milk, hence the earlier name lactoflavin. Note that like vitamin B_1, riboflavin has a pyrimidine nucleus. Riboflavin is widely distributed in very small amounts in most animal and plant material. It suffers little destruction by cooking. No rich natural source is known. The daily requirement for man is about 2–3 mg.

P-P Factor (Nicotinic Acid, Niacin)

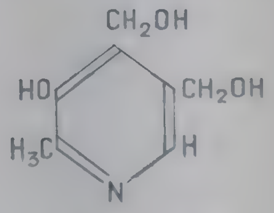
The pellagra-preventing (P-P) factor is now quite distinct from riboflavin. In the past the P-P factor was called B_2 (G in America). The chief signs of pellagra are a characteristic dermatitis, especially where the skin has been exposed to light (hence the expression " pellagra glove ") and pronounced digestive disturbances and wasting. There has been much controversy as to the cause of the disease, which has been attributed to a vitamin deficiency, lack of suitable protein, and also to a toxic substance formed in maize by certain micro-organisms. The incidence of the disease is almost confined to districts in which maize is the staple food, *e.g.*, the " corn belt " of U.S.A., Southern Russia, Egypt, and northern Italy. Seven thousand died of pellagra in U.S.A. in 1930 out of 120,000 cases. The diet in such districts is obviously poor, *e.g.*, in the " corn belt " it is chiefly maize and white rice with beans, molasses, and a little salt pork. Such a diet, unless supplemented by meat, milk, eggs, etc. (which the workers are frequently unable to afford), produces pellagra in six to eight months. The disease is a consequence of poverty, and not ignorance. Of eleven con-



NICOTINIC ACID



NICOTINAMIDE



PYRIDOXIN

victs volunteering to live on such a diet, five went down with the disease in five months. Recent experiments on dogs with " black tongue," the canine equivalent of pellagra, showed that they could be cured by giving nicotinic acid or its amide.

Similar results were obtained on pigs and monkeys; the successful treatment of several cases of human pellagra has also been recorded, suggesting that the disease is really a vitamin deficiency, and that the vitamin is nicotinic acid or its amide.

Nicotinamide was identified as part of two co-enzymes concerned in oxidation-reduction reactions before its isolation as a vitamin (p. 129, 130).

The richest natural sources of nicotinic acid or amide are rice polishings and yeast. Good sources are liver, kidney, lean meat and peanuts, while milk, fish, cereals and potatoes contain appreciable amounts.

Pyridoxin (Vitamin B_6, Adermin)

Lack of this vitamin in the rat causes a characteristic dermatitis of the extremities (acrodynia). The function of pyridoxin in human metabolism is not yet clear. It has been used with success for treatment of certain conditions exhibited by pellagrins which do not respond to administration of nicotinic acid or riboflavin. Pyridoxin has been identified as 3-hydroxy-4 : 5-di(hydroxymethyl)-2-methylpyridine. The richest natural sources are egg yolk and vegetable fats, particularly wheat germ oil. See also tryptophan, p. 281.

Pantothenic Acid

The fourth compound identified in the " vitamin B_2 complex," pantothenic acid, was first studied as a factor preventing dermatitis in chicks. Little is known of its importance in human nutrition. In cases of beri-beri, ariboflavinosis and pellagra blood values below normal (19 to 32 μg. per 100 c.c.) have been observed. Pantothenic acid is a peptide-like compound of β-alanine and $\alpha\gamma$-dihydroxy-$\beta\beta$-dimethylbutyric acid.

$$HO.CH_2.C(CH_3)_2.CH(OH).CO.NH.CH_2.CH_2COOH$$

It is very soluble in water, and widely distributed in small amounts.

Vitamin C (Ascorbic Acid)

Function. The absence of vitamin C from human dietary causes scurvy in three or four months. It is therefore called the antiscorbutic vitamin. Prominent signs of this disease are general weakness, swelling of the gums and loosening of the teeth, many small hæmorrhages into the skin and mucous membranes, and brittleness of bones. If not soon treated the disease is fatal. In the absence of vitamin C the capillary walls become fragile or abnormally permeable, causing extravasation of blood into the tissues. Measurement of capillary resistance has, in fact, been used to assess mild deficiency of vitamin C. This increased capillary fragility may be the cause of some of the symptoms of scurvy.

Lack of vitamin C may result in damage to tooth structure due to partial degeneration of odontoblasts and other cells, and in this way predispose to dental caries. The effect of vitamin C deficiency on the histological structure of guinea-pigs' teeth has been applied for the assay of antiscorbutic activity. There is evidence that vitamin C is also necessary for the health of the gums and protects against pyorrhœa.

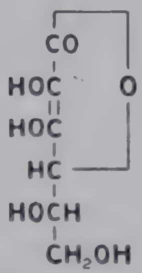
The chemical nature and wide distribution of vitamin C suggests that it may play a part in oxidation-reduction reactions. There is a high concentration of the vitamin in the aqueous and vitreous humours of the eye, but its function there is unknown.

The treatment and prevention of scurvy by supplementing the diet with substances now known to be potent sources of vitamin C have long been known. There are frequent records of epidemics of scurvy from the Crusades of the thirteenth century to the 1914–1918 War. A Spanish galleon in the fifteenth century was found drifting with the whole crew dead from scurvy. By the end of the sixteenth century the value of lemon juice was known but not universally believed. Commodore Lancaster in 1600 protected his crew with lemon juice and had to send some of his men on board three accompanying ships to set their sails owing to the disablement of their crews by scurvy. A monograph on scurvy by the naval physician, Lind, quotes the opinion of the surgeon of the Austrian Army in 1720 that lemon juice and fresh green vegetables were the only agents effective in curing scurvy. It was only after the safety of the Channel Fleet had been endangered by epidemics of scurvy that the Admiralty ordered regular supplies of lemon juice for the British Navy in 1800, with the result that there was a remarkable decrease in the incidence of the disease. Scurvy is prevalent in times of war or famine ; outbreaks occurred in the siege of Paris, the Russo-Japanese War and the War of 1914–1918.

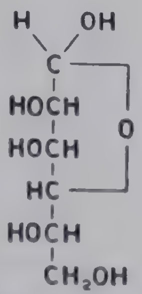
Not all animals are susceptible to scurvy. The rat, rabbit, dog and birds do not get scurvy on a vitamin C deficient diet ; man, apes, monkeys and guinea-pigs are very susceptible. (This is why the guinea-pig is used as the experimental animal for the study of the vitamin.) Since all species so far examined contain in the suprarenal glands a considerable amount of vitamin C which is diminished only in the susceptible species when there is a dietary deficiency, it is concluded that non-susceptible animals have the power of synthesising the vitamin.

Evidence has accumulated to show the importance of an adequate intake of vitamin C for the promotion of normal healing of wounds and fractures in both man and the guinea-pig. A lowered intake of vitamin C may result in a scar of low tensile strength with risk of post-operative reopening of wounds and delayed healing of fractures. It has been estimated that for man an intake of 40 mg. of ascorbic acid per day is required to secure adequate healing of wounds ; less than 20 mg. per day may cause scars of low tensile strength to form.

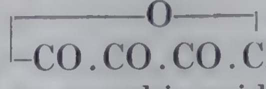
Chemical Nature. Vitamin C has the curious history of having been isolated in the pure state before it was realised that it was the vitamin. It was first isolated by Szent-Györgyi in 1928 from suprarenal glands, and subsequently from orange juice and cabbage, as the substance responsible for the powerful reducing action of the suprarenal cortex. It was not until 1932 that it was discovered to be the most powerful antiscorbutic substance known, although it had been known that vitamin C concentrates were in some way associated with reducing properties. The discovery stimulated an intense chemical interest, as a result of which the vitamin was identified and synthesised in 1933 and named *ascorbic acid*. The formula shows ascorbic acid as a derivative of a hexose called *l*-gulose.



(VITAMIN C
ASCORBIC ACID)



l-GULOFURANOSE

The natural acid is the *l*-variety which is more active than the *d*-. Ascorbic acid is a white crystalline substance with powerful reducing properties ; it reduces Fehling's solution in the cold. It is very readily inactivated by oxidation. The first stage is oxidation to dehydroascorbic acid,  $\overbrace{\text{-CO.CO.CO.CH}}^{\text{O}}\text{.CHOH.CH}_2\text{OH}$, which is biologically equivalent to ascorbic acid since it is readily reduced to ascorbic acid in the tissues. But dehydroascorbic acid is very easily oxidised irreversibly, especially in the presence of traces of copper salts or by heat, to products having no antiscorbutic activity. Ascorbic acid can be estimated on the basis of its reduction of an indicator, 2 : 6-di-chlorophenol-indophenol.

Sources. The chief dietary sources of vitamin C are fresh fruit and green vegetables. Lemons, oranges, tangerines, grape fruit, black currants, tomatoes, lettuce, cress, paprika, cauliflower and cabbage are excellent sources if fresh. Lime juice has a variable content and may be almost devoid of activity ; this was the cause of the outbreak of scurvy in the North Pole Expedition of 1875 which relied upon lime juice as an antiscorbutic. Cereals and other seeds are devoid of vitamin C, but form it on germinating ; this provides a very convenient form for the transport of antiscorbutic substances, and was used in the British Expeditionary Force in Serbia in 1918. The coincidence of an epidemic of scurvy in Great Britain with the potato famine of 1917 suggests that the potato, although not a rich source of vitamin, is of considerable dietetic importance in this respect. On the restricted diet imposed by the recent war the potato provided as much as 40% of the total ascorbic acid ingested. Freshly killed meat is an effective source of vitamin C, but its value is greatly reduced after hanging and cooking. The antiscorbutic value of fresh raw fish (but not cooked) has been proved in Polar expeditions.

About the beginning of this century bottle feeding of babies became fashionable. The artificial foods or sterilised milks used were practically devoid of vitamin C so that there was a high incidence of infantile scurvy (Barlow's disease). Now, even breast-fed infants are given a supplement of orange juice. Human milk contains an adequate amount of vitamin C if the mother's intake is adequate ; cow's milk does not contain sufficient.

Vitamin C is stable below pH 6·8 at room temperature but readily oxidised in an alkaline medium. In fruits and vegetables oxidation sets in slowly as soon as the crop is gathered owing to the action of an enzyme. This oxidation is accelerated if the material is cut up or minced, and very greatly accelerated by the rise in temperature in the early stages of cooking, when the greatest destruction of the vitamin occurs. Much vitamin may be lost in the water during boiling; there is, however, usually less loss in the cooking of fruits than of vegetables since the water from the former is consumed. Many cooked fruits and vegetables slowly lose vitamin C on keeping even at room temperature. An apparent increase in vitamin C content has been observed when certain vegetables and fruits are cooked. This has not been satisfactorily explained.

In modern canning processes vitamin C is better preserved than in domestic cooking because the crop, which must be of the best quality, goes direct to the factory without market delays, the volume of water used is relatively small, and copper is now rigorously excluded from the cooking vessels.

Vitamin P (Citrin, Eriodictyol)

It has been reported that the hæmorrhages of human scurvy are not cured by pure ascorbic acid alone, but that another factor, citrin or vitamin P, present in lemon juice and paprika, is essential. While there has been some clinical support for this, the existence of the vitamin cannot be regarded as definitely established.

For the occurrence of vitamins in foods, see p. 436, and in urine, p 454. For the daily requirements of vitamins, see p. 400.

PART III

CHAPTER XXIX

THE ENERGY REQUIREMENTS OF THE BODY (1, 3, 5, 8, 60)

THE ultimate chemical function of a living animal is to use food and oxygen to maintain its tissues and temperature and to provide energy for work. In a starving animal this function is performed at the expense of its own tissues, which in consequence waste ; in an adult animal which is in equilibrium and maintains a constant weight, the food intake provides a measure of this function. The energy of the body is derived ultimately from the oxidation of the food since, in general, hydrolytic reactions involve only small energy changes. Actually a small part of the daily energy output would be due to breakdown of the animal's own tissues, but since this would be repaired by a corresponding amount of food the ultimate effect is that in an animal *in perfect equilibrium* the energy output corresponds to the amount of food consumed. The potential energy of the food provides the *actual* or *kinetic* energy of the body which is mainly in the form of heat and work, since very little energy is expended in the production of sound, etc. The potential energy of the food is the heat given out when the food is completely burnt to carbon dioxide, water and nitrogen ; this is greater than the energy which the animal derives from the food because it excretes matter which still has considerable potential energy, *e.g.*, urea and other nitrogenous compounds. The animal does not form free nitrogen. In this chapter we shall consider the energy needs of man and how these needs can be determined, or in other words, how much food in terms of Calories he should take each day ; in Chapter XXX we shall make certain stipulations as to the quality of the food.

THE POTENTIAL ENERGY OF FOOD

The potential energy of a foodstuff can be found by measuring the heat produced when a given amount is completely burnt in oxygen in a calorimeter. In practice, in order to provide sufficient oxygen for complete combustion in a calorimeter small enough to make for accurate measurement of its heat output, the oxygen is put in under considerable pressure. This requires a calorimeter of very robust construction and has given rise to the term *bomb calorimeter*. The energy values, as Calories,* of most foodstuffs have now been determined and are available in tables in dietetic handbooks.

The energy value can also be determined indirectly by burning the food in oxygen in a closed circuit apparatus (*oxycalorimeter*) and measuring the oxygen consumed. The Calories are calculated from the oxygen consumption by factors previously determined in the bomb calorimeter.

One gram of a monosaccharide yields 3·75 Calories, while a disaccharide yields 3·95 and starch 4·23 Calories per gram. Hence we take 4·1 as the average Calorie yield of 1 g. of mixed carbohydrate such as we should eat. The average values for fat and protein are 9·3 and 5·4 respectively. Normally carbohydrate and fat are completely oxidised in the body to CO_2 and H_2O under equilibrium conditions so that we can take their potential energy values as being the physiological energy values. Protein, however, is never completely oxidised in the tissues, since part is excreted (as urea, etc.) in urine. By subtracting the potential energy value of the excreta per gram of protein metabolised, an average value of about 4·1 Calories is obtained for mixed proteins. Animal protein has a higher physiological energy value (4·3) than has vegetable protein (4·0). The average physiological energy values of the three proximate principles of our food are, then :—

Carbohydrate	4·1 Calories per gram.
Fat	9·3 ,, ,,
Protein	4·1 ,, ,,

Using such figures as a basis for the calculation of the physiological energy value of foods, remarkable agreement has been obtained experimentally between a man's energy income and output, indicating that energy derivable from the combustion of food is the same whether burnt at once, as in the bomb calorimeter, or in stages as in the body.

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* The unit of energy used to express the needs and output of the body is the large Calorie (with a capital C). This is the amount of heat required to raise 1 kg. of water from 15° C. to 16° C. It is equal to 1,000 small or gram calories, or $4·2 \times 10^{10}$ ergs.

THE ENERGY REQUIREMENT OF MAN

The energy needs of a man can be determined either directly by measuring his heat output in a calorimeter or indirectly by measuring his oxygen consumption.

(1) **Direct Method** (57). This is the most accurate method, but has the disadvantage that the determination is time-consuming and laborious as well as demanding very expensive apparatus. The subject is placed in a calorimeter equal in size to a small room and arranged so that he can take his meals, do any suitable work and sleep on a bed included in the furnishing of the calorimeter.

In the **Atwater-Benedict calorimeter** (Fig. 24) there are quadruple walls (and floors and ceilings) to the room ; the innermost copper wall is surrounded by a zinc * wall which is enclosed by a double wall of wood. Each wall is separated from the others by an air space. The zinc wall is equipped with electric resistance wires and pipes through which water can be circulated. In this way it can be heated or cooled at will, and so kept at the same temperature as the copper wall (both walls are liberally fitted with thermocouples recording the wall temperatures). So long as the temperature of the outer wall is the same as that of the inner, there will be no loss or gain of heat from the inner room.

The temperature inside the calorimeter is kept practically constant by passing a current of water through pipes. This removes heat lost by the subject through radiation or conduction ; this heat is calculated from the measurement of the rate of flow and the temperatures of the water entering and leaving the calorimeter. A considerable amount of heat is expended in evaporating water in expired air and perspiration. This is measured by absorbing the water from the circulating air by sulphuric acid, weighing it, and multiplying by its latent heat of vaporisation.

Ventilation is attained by a continuous circulation of the air in the calorimeter through vessels which remove water and CO_2 (by soda lime) ; oxygen in measured amount is introduced at intervals to replace that used. The temperature of the air entering and leaving the calorimeter is recorded and adjusted if necessary. A tension equaliser prevents changes of pressure in the circuit. Provided the subject does not keep near the inlet pipe, no ill-effects are felt from the introduction of dry air, the water vapour expired causing a sufficient humidity in the calorimeter.

A porthole with airtight doors which can be opened one at a time provides a means of passing in food and passing out excreta without appreciable heat loss.

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* Zinc was used instead of copper because it was cheaper

So carefully have the technical details been attended to that a quantity of alcohol burnt in the calorimeter gives a value almost identical with that found in the bomb calorimeter.

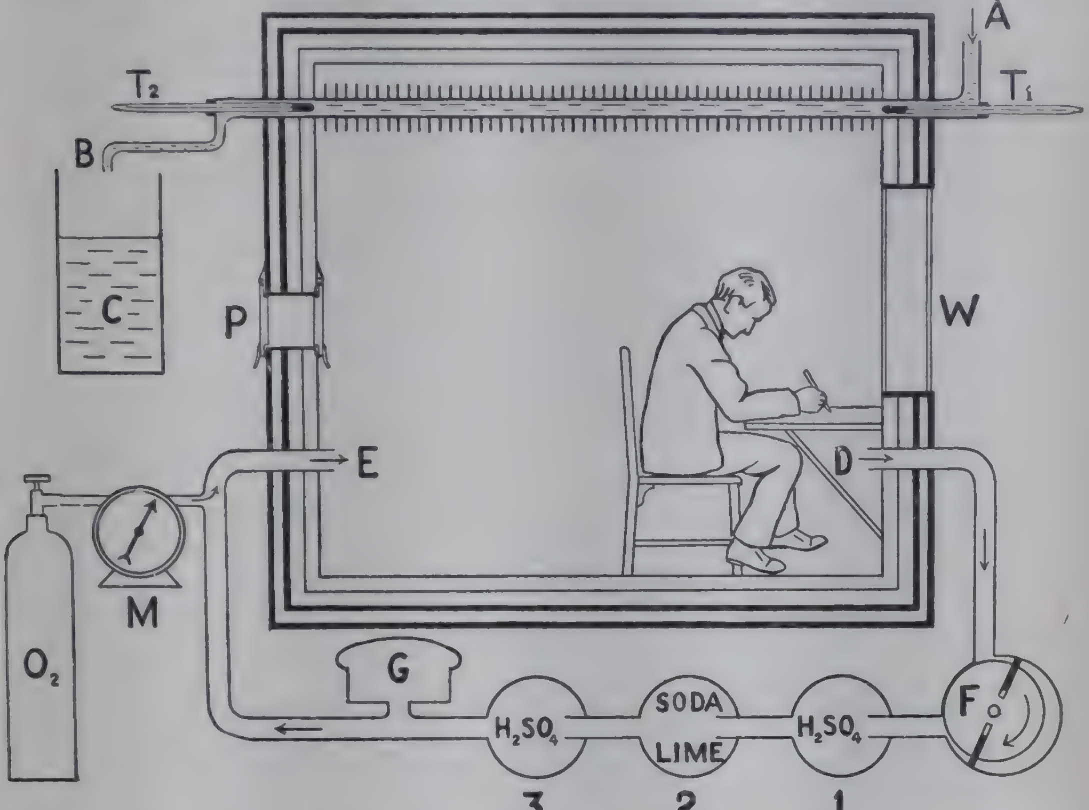

FIG. 24. Diagram of Atwater-Benedict respiration calorimeter with subject engaged in sedentary work.

Walls constructed as described in text.

A to B, heat-absorbing system. Temperature of water is recorded by thermometers T_1, T_2. Flow of water is measured by weighing the water collected in can C.

D to E, ventilating system. F, rotary blower to keep air circulating. Water given off in expired air is absorbed by H_2SO_4 (1) and CO_2 by soda lime (2). Moisture given off by soda lime is trapped by H_2SO_4 (3). G, tension equaliser. Oxygen from the cylinder, O_2, is measured by the meter, M, before entering the circuit.

P, porthole for entry of food and removal of excreta.

W, window, sealed after the subject has entered.

(2) **Indirect Methods.** Indirect methods of determining the energy output depend upon finding the oxygen consumed and carbon dioxide produced under the given conditions. The ratio $\frac{\text{Volume of } CO_2 \text{ formed}}{\text{Volume of } O_2 \text{ used}}$ is known as the **Respiratory Quotient,**

abbreviated to R.Q., and varies according to the substances being metabolised. In the combustion of carbohydrate the volume of CO_2 formed equals that of the O_2 used, so that R.Q. = 1.

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O \quad \therefore \text{R.Q.} = \frac{6}{6} = 1.$$

With fat (triolein) the R.Q. is 0·71, thus

$$C_{57}H_{104}O_6 + 80\ O_2 = 57\ CO_2 + 52\ H_2O \quad \therefore \text{R.Q.} = \frac{57}{80} = 0{\cdot}71.$$

The R.Q. of protein cannot be calculated so easily because protein is not completely oxidised in the body. By subtracting the portions of a protein which are excreted, the residue undergoing oxidation is found and hence the amount of oxygen required for, and the CO_2 formed by its combustion can be calculated. In this way the R.Q. of proteins is found to be 0·8–0·82. The R.Q.'s for the physiological oxidation of the three chief food constituents are therefore :—

Carbohydrate	1·0
Fat	0·71
Protein	0·81

On an ordinary mixed diet the R.Q. is approximately 0·85. Chinese and Hindoos who live on a high carbohydrate diet (rice) have R.Q.'s above 0·9. Under certain conditions values outside the range 0·71–1·0 are observed. This is due to interconversion reactions. Geese being fattened were observed to have R.Q. 1·38 due to conversion of carbohydrate into fat in which CO_2 would be formed without consumption of oxygen, *e.g.*,

$$13\ C_6H_{12}O_6 \rightarrow \underset{\text{(Oleostearopalmitin)}}{C_{55}H_{104}O_6} + 23\ CO_2 + 26\ H_2O$$

The conversion of glycerol into sugar, which probably occurs in diabetics and hibernating animals, would lower R.Q. since oxygen is used but no CO_2 produced

$$2\ C_3H_8O_3 + O_2 = C_6H_{12}O_6 + 2\ H_2O.$$

The conversion of certain amino-acids into sugar in diabetics requires oxygen and lowers the R.Q. Since, in addition, carbohydrate metabolism is deficient in a diabetic, quotients below 0·7 may be observed in severe cases.

The indirect methods of determining energy output are of two types. In one the subject breathes the same nitrogen all the time, while oxygen is added to replace that used and CO_2 is removed as formed (*closed circuit methods*); in the other the subject breathes ordinary air and only his expired air is collected (*open circuit methods*).

Closed Circuit Methods. To obtain accuracy comparable with that of the Atwater-Benedict calorimeter a determination lasting at least twenty-four hours is necessary so that the subject's urine may be collected and analysed. He is confined in a small gas-tight room (respiration chamber, Fig. 25), through which air is circulated under conditions similar to those in the Atwater-Benedict apparatus, except that the air before entry is allowed to take up water vapour. The following numerical example shows how his energy output could be calculated.

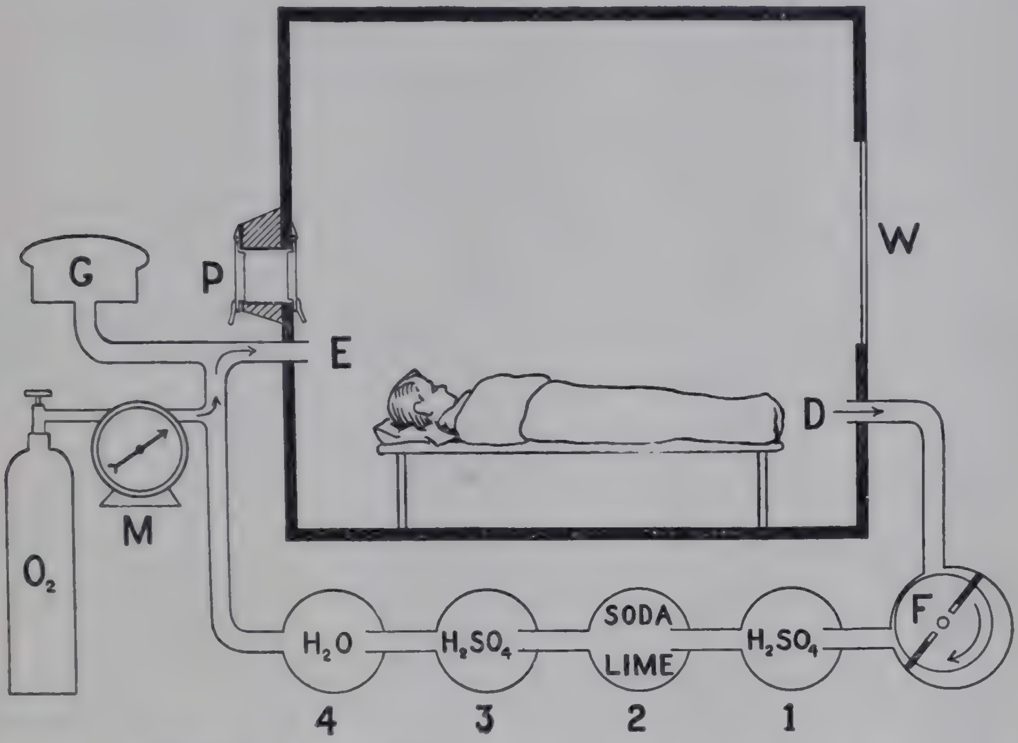

FIG. 25. Diagram of a respiration chamber with subject lying down in bed. D to E, ventilating system. F, rotary blower to keep air circulating Water given off by expired air is trapped by H_2SO_4 (1). Expired CO_2 is absorbed by soda lime (2). Moisture given off by soda lime is trapped by H_2SO_4 (3). Humidity is restored by passing the air over water in (4). Oxygen from cylinder, O_2, is measured by meter, M, before entering the circuit. G, tension equaliser.

P, porthole for entry of food and removal of excreta.

W, window, sealed after the subject has entered.

During twenty-four hours the subject used 450 litres of oxygen and put out 380 litres of CO_2. His urine contained 13·5 g. of nitrogen.

With average protein, 1 g. urinary N corresponds to 6·25 g. protein metabolised. This amount of protein requires 5·923 litres O_2 and produces 4·754 litres CO_2. Hence 13·5 g. urinary N has involved the consumption of $13·5 \times 5·923 = 80$ litres O_2 and production of $13·5 \times 4·754 = 64$ litres CO_2.

We have, therefore,

	O_2 used	CO_2 formed
Total metabolism . . .	450 litres	380 litres
Protein metabolism . . .	80 ,,	64 ,,
Carbohydrate + Fat metabolism .	370 ,,	316 ,,

$$\therefore \text{ The carbohydrate + fat, or non-protein, R.Q. is } \frac{316}{370} = 0·85.$$

From this it can be calculated, although tables (see Refs. 1, 5, 8 or 61) compiled for the purpose are generally used, that a non-protein R.Q. of 0·85 corresponds to the oxidation of 0·580 g. carbohydrate and 0·267 g. fat per litre of oxygen used. Hence during the twenty-four hours the subject used

$370 \times 0·580 = 214$ g. carbohydrate.
$370 \times 0·267 = 99$ g. fat.
$13·5 \times 6·25 = 84$ g. protein.

Hence, using the Calorie values given on p. 369, his energy output is

$$(214 \times 4·1) + (99 \times 9·3) + (84 \times 4·1) = 2{,}140 \text{ Calories.}$$

For clinical purposes this method is modified and shortened. The subject breathes through a mouthpiece or mask an air circuit in which provision is made for absorption of CO_2 and measurement of oxygen consumption. The oxygen is usually contained either in a gasometer (Benedict, Knipping), or floating hinged tank (Krogh), and measured by the descent of the gasometer bell or tank into the water, the descent usually being automatically recorded by a pen or pointer which writes on a drum. These instruments are therefore called **recording spirometers** (Fig. 26). The duration of the experiment depends on the amount of oxygen which can be put in; it is frequently only ten or fifteen minutes. In the Knipping apparatus CO_2 is absorbed by KOH and subsequently liberated by H_2SO_4 and measured; in the others the CO_2 is absorbed by soda lime without measurement and the patient's non-protein R.Q. is assumed to be 0·82. In these short experiments protein metabolism cannot be measured separately. Tables (see Refs. 1, 5, 8 or 61) are available which give the Calories generated by the utilisation of 1 litre of oxygen at a given non-protein R.Q. It is customary to neglect the error (about 2%) due to not estimating protein metabolism. In the example quoted above the non-protein R.Q. was 0·85, which is equivalent to 4·862 Calories per litre of oxygen used. Hence, neglecting protein metabolism, the energy output would be 4·862 × 450 = 2,180 Calories in twenty-four hours, compared with 2,140.

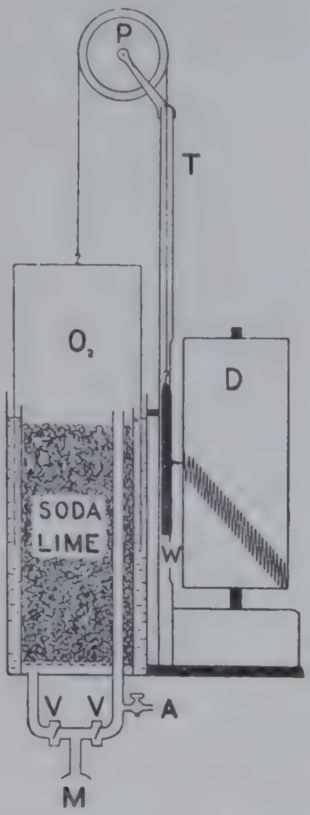

FIG. 26. Diagram of recording spirometer (Benedict type).
The subject breathes through the mouthpiece or mask, M, fitted with valves, V, V. The amount of O_2 used is recorded on the rotating drum, D, by the writing point attached to the counterbalance weight, W, of the gasometer. The tube, T, acts as a guide for the weight, W, and supports the pulley, P. The gasometer is filled with O_2 through the tap, A, at the beginning of the determination. Expired CO_2 is absorbed by the soda lime.

Open Circuit Methods. These methods are more accurate than the simplified closed circuit methods, since both oxygen and CO_2 are determined, although the protein error is still present. The subject is fitted with a mouthpiece or mask equipped with valves so that ordinary air is inspired, but expired air is collected either in a rubber canvas bag (Douglas) (Fig. 27) or a gasometer (Tissot). The volume of expired air is measured and a sample analysed in Haldane's gas analysis apparatus for oxygen and CO_2. Owing to the skill necessary for these analyses, the simpler closed circuit methods are usually preferred clinically. In the open circuit method the difference in the percentages of oxygen in the inspired and expired air multiplied by the volume of expired air per minute (after correction for any change in the nitrogen percentage) gives the oxygen consumption per minute. The CO_2 production is similarly found and hence the R.Q. The Douglas method (Ref. 61) is most frequently employed experimentally because it has the advantage over all other methods that the Douglas bag can be carried on the subject's back while he is doing work, such as running upstairs or climbing a ladder.

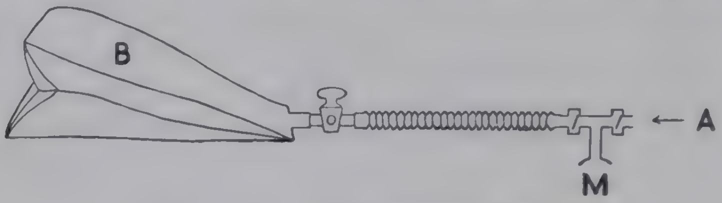

FIG. 27. The Douglas bag.
The subject breathes through the mouthpiece or mask, M. He inspires room air, A, and expires into the bag, B.

Basal Metabolism

The energy output of an individual is affected by many factors, even if he does no physical work. These factors are minimised if the subject is kept lying down and still in bed, covered by bedclothes to prevent heat loss, and neither " too hot " nor " too cold " ; he must be in repose both mentally and physically, and in the post-absorptive state, *i.e.*, sufficient time must have elapsed since the last meal for digestion and absorption to have ceased ; his breathing must be involuntary and his temperature normal. His energy expenditure under these conditions is known as his *basal metabolism*, and represents the heat required to maintain his body temperature and the energy necessary for the respiratory movements and contractions of the heart. Since the surface of a small animal is greater per unit weight than that of a large one, its heat loss will be greater ; it will therefore have a relatively greater basal metabolism. The basal metabolism is proportional to the surface area of an animal, and so it is customary to compare basal metabolisms in terms of the Calories produced per square metre body surface per hour. This value is called the **basal metabolic rate** and abbreviated to B.M.R.

Surface area can be determined directly by covering the subject with close-fitting thin underwear which is impregnated with wax and reinforced with paper strips. The cast so obtained is cut up into small pieces which are spread out and photographed on to paper of uniform thickness. The areas are then carefully cut out and weighed. Such a method is obviously very laborious, and in practice formulæ (*e.g.*, that of Du Bois, $A = W^{0 \cdot 425} \times H^{0 \cdot 725} \times 0 \cdot 007184$ sq. m.) correlating weight (W) and height (H) with surface area (A) are used, usually in the form of nomograms or charts arranged so that a line joining points representing the subject's weight and height intersects a line giving the correct surface area (see Refs. 5 or 8). The normal basal metabolic rate of a man in his prime is about 40 Calories per sq. m. per hour, *i.e.*, nearly 1,000 Calories per sq. m. per day. For women the value is about 37·5 Calories. The normal standard values vary not only with sex but with age. Racial variations, probably partly due to dietary and activity habits, are also observed.

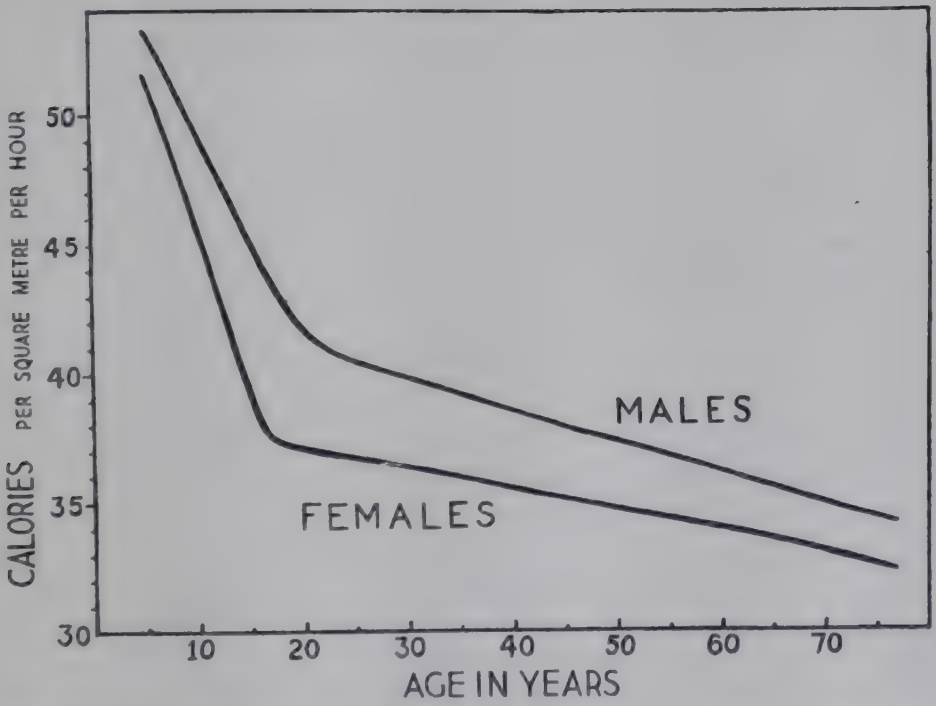

FIG. 28. Normal standards for basal metabolic rates at different ages. (Drawn from Boothby and Sandiford's figures.)

The basal metabolic rate of the new born is near that of the adult but it increases in the first few months of life, attaining a maximum between three and four years.

Clinically the observed value for the B.M.R. is compared with the standard value predicted by the patient's sex, weight and height.* In health, departures of more than 15% are rare. In pathological conditions, particularly thyroid diseases, big departures may be seen, *e.g.*, exophthalmic goitre frequently 50% high, myxœdema 20–30% low. An increase of 1° F. in body temperature causes a rise in B.M.R. of about 7% (1° C. = 13%) so that a high B.M.R. is observed in fevers. Mental excitement increases basal metabolism ; this is a big technical difficulty in determining the B.M.R. of an excitable patient.

Effect of Food on Basal Metabolism (58, 59)

The finding that a man's basal metabolism is, say, 1,800 Calories per day does not mean that he only needs food equivalent to 1,800 Calories per day to maintain himself if he spends the day in bed without doing any work. Even if completely digested food were injected intravenously his output would exceed 1,800 Calories by about 200 Calories. This means that the excess Calories would have to come from his own tissues, and that to maintain his weight he would have to take over 2,000 Calories. If he persisted in taking 1,800 Calories he would lose weight until he was of such a size that 1,800 Calories was sufficient to keep him in equilibrium ; by then his basal metabolism would have fallen to nearly 1,600. Careful experiments have made it clear that the increased energy output after taking food is due solely to a specific stimulating effect of food on metabolism, and not to any work done in digestion or absorption or to a specific stimulant in food, for the effect is obtained by intravenous injection of pure glucose or amino-acids. This effect is known as the **Specific Dynamic Action** of foodstuffs, and abbreviated to S.D.A. It is most marked with proteins, which cause an increase of 30%, *i.e.*, an animal with basal metabolism of 100 Calories fed on protein equivalent to 100 Calories would show an energy expenditure of 130 Calories. Fat has a S.D.A. of 4% and carbohydrate 5–6%. A mixed diet is usually taken as having a S.D.A.

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* For table relating standard O_2 consumption to height and weight see Ref. 3.

of 10–12%. This means that if an animal with a basal metabolism of 100 Calories were given daily 100 Calories of food, its heat production on the first day would be 112 Calories, of which twelve would be obtained at the expense of its tissues; these would continue to waste until its basal metabolism had fallen to such a level (90 Calories) that its basal metabolism + S.D.A. = 100 Calories. Therefore, merely to maintain the body in equilibrium without doing any work, food must be supplied to the extent of 12% over the basal energy requirement.

The reason for the specific dynamic action of foodstuffs is not known. Amino-acids differ in the extent of their S.D.A., those most active in this respect being glycine, alanine (50%), leucine, phenylalanine and tyrosine. The absence of a specific dynamic effect of protein after hepatectomy suggests that the phenomenon is a result of metabolism of amino-acids in the liver. Consideration of the amino-acids quoted shows that effect is not related to the glucogenetic properties of the amino-acids.

Effect of Work on Metabolism (60, 65, 68, 69, 70)

Work is performed at the expense of increased metabolism, so that in order to maintain the body in equilibrium, additional food proportional to the work done is necessary. If extra food is not supplied, this energy must be derived from the tissues. From experiments by the direct method Atwater found that the performance of mechanical work equivalent to 1 Calorie (= 426 kg. m.) involved an additional heat elimination of approximately 5 Calories, *i.e.*, the mechanical efficiency of the human body is about 20%, comparable with that of a petrol engine. This figure may be improved by training, since a given task can be performed with less muscular exertion by one who has learnt the easiest way of doing it. The rate at which work is done is also a factor in the energy expenditure; a slow steady output of work is usually more economical, *e.g.*, less energy is required for walking a mile than running.

The energy for work appears to be derived *immediately* from combustion of glycogen in the muscles, since the sudden performance of work is coincident with a raised R.Q. In very severe exercise an R.Q. of 0·98 may be found (resting 0·83). *Ultimately* work is done at the expense of the food, and the R.Q. over twenty-four hours is proportional to the amounts of carbohydrate, fat and protein consumed (assuming the man is not wasting or fattening), regardless of the work done. The net result is as if carbohydrate, fat and protein were used to provide energy. Whether they are used directly or after conversion (*e.g.*, fat or protein to carbohydrate) is not known. Protein does not appear to be used for muscular exercise if an adequate amount of carbohydrate and fat is available, since the urinary nitrogen is no greater after a day of exercise than after a day of rest, although there is ample evidence that several amino-acids can readily form glucose (p. 272).

Foodstuffs are not strictly interchangeable on the basis of their energy values (apart from the qualitative stipulations of Chapter XXX) as was once thought (Rubner's isodynamic law). Krogh showed that a given amount of work which was performed by a man at the cost of 100 Calories of carbohydrate required 110 Calories of fat. It has been suggested that the extra 10 Calories represent the cost of conversion of fat to carbohydrate, although no chemical evidence of this change has been found (see p. 294). Carbohydrate is more effective than fat in sparing protein. Since, in addition, it is cheap and easily digested, carbohydrate is regarded as the best food for providing extra energy.

Effect of Temperature on Metabolism

An adult must take sufficient food to compensate for his heat elimination. This will vary not only with the amount of work performed, but also the external temperature. The body maintains its temperature by the heat produced in the combustion of foodstuffs. Under ordinary circumstances the heat generated in muscular exercise can be used for this purpose. Shivering involves the involuntary production of heat by muscle contraction. If an individual were exposed to cold without doing work, allowance would have to be made for extra food to provide heat to keep up his temperature. The energy of specific dynamic action can contribute to heat regulation, but probably cannot be used for muscular work. Heat elimination is also affected by conditions affecting the evaporation of water, *e.g.*, the humidity of the air and winds.

A man's food requirements will therefore depend upon his basal metabolism, specific dynamic action, the work to be done and the climatic conditions. That is for a man in equilibrium. A starved man requires more to build up his tissues, a pregnant woman more for the fœtus and additional tissue, but, greater than any, a child requires additional food for growth out of all proportion to its basal metabolism.

So many factors would have to be considered for the accurate calculation of the quantity of food needed for an individual in a given occupation that values have been obtained either by direct measurement in a calorimeter or by statistics of the diets which have been found satisfactory by experience. A man of 11 stone (70 kg.) and 5 ft. 11 in. (180 cm.), aged 30, would have a basal metabolism of 1,800 Calories per day. (His surface area would be 1·87 sq. m. and B.M.R. 40.) The allowance for S.D.A. would be 200 Calories. If engaged in a sedentary occupation he would require a total of 2,500–2,800 Calories, *i.e.*, he needs 500–800 Calories for work, including walking to and fro. Rough figures for the total daily requirements of different grades of work are :—

Grade	Lowest Calorie Requirement (24 hours)	Example of Trade
Sedentary . . .	2,500	Tailors, shoemakers.
Light work . . .	3,000	Carpenters, painters.
Moderate work . .	3,500	Farmers, soldiers.
Heavy work . . .	4,000	Stonemasons, navvies, lumbermen.

Very heavy work may entail the expenditure of 8,000 Calories (*e.g.*, lumbermen). Under exceptional conditions, *e.g.*, long bicycle races, 10,000 Calories have been used in twenty-four hours. The food requirement of the average working man is usually taken as 3,000 Calories per day, for which 3,400 Calories should be purchased to allow for waste in preparation and digestion.

A more accurate estimate of a day's requirement can be drawn up if details of the individual's activities are known. The following table gives the Calories expended per hour at various occupations. From these data the energy expended during the day can be calculated. By adding this to the individual's basal metabolism for 24 hours we get the daily requirement. Strictly, 5 per cent. should be addad to the total to allow for specific dynamic action.

Calories Expended Per Hour in Various Activities

(Data from Ref. 64).

Sitting at rest	30	Peeling potatoes	40
Standing	40	Dish washing	70
Dressing or undressing	50	Typewriting	40
Walking 2·6 m.p.h.	140	Writing	30
,, 3·75 m.p.h.	240	Tailoring	75–84
Running	500	Painting furniture	100
Swimming 2 m.p.h.	550	Carpentry	137–176
Cycling	175	Metal work	180
Driving car	60	Blacksmith	276–351
Dancing, foxtrot	240	Stone-masonry	330
Boxing	800	Sawing wood	400
Sewing	40	Laundress	124–214
Sweeping floor	100	Coal cutting	103–138

Note : These are average figures. The actual energy expenditure will, of course, depend on the weight of the individual and the facility with which he does a given task. One person may use up more energy than another by unnecessary movements.

Consideration of one's own habits will show that our food intake may vary from day to day. Most of us make Sunday a high Calorie day. When the average consumption at a residential college was recorded over six consecutive days it was found that Sunday's consumption was 36% above the average for the six days.

Women have a lower basal metabolic rate (7%–10% lower) than men, and are smaller in size, so that their average calorific needs are less. The average Englishwoman, aged 30, weighing 9 stone and 5 ft. 3 in. high, has a surface area of 1·56 sq. m. and B.M.R. 37, and hence a basal requirement of 1,440 Calories per day, or 1,600 with S.D.A., so that if engaged in a sedentary occupation she would require food to the value of 2,200 Calories. The usual estimate of the average working woman's requirement is 2,500 Calories (2,800 purchased), *i.e.*, about 83% of a man's requirement.

A glance at the curve on p. 377 will show that children have a much higher basal metabolic rate than have adults and that, consequently, they will require food out of all proportion to their size, for their muscular activity is usually great. A statistical review of the dietary of children in well-to-do American homes (Holt and Fales, 1921) shows a peak intake at the age of sixteen in both boys and girls with Calorie values (4,090 and 3,160) considerably in excess of adults (3,265 and 2,640). Unfortunately a similar series for English children is not available, although what evidence there is suggests that lower values are more likely. It must be clear that the relation depends upon the work done by the adult. In a well-to-do home, the adults are not usually engaged in manual labour, and may well require less food than adolescents. A navvy, however, would always require more than his son. Recommendations, based upon computations and not statistical analyses of actual approved dietaries, are :—

VALUES RECOMMENDED FOR GREAT BRITAIN

Age	Cathcart and Murray (M.R.C., 1931, Ref. 70)		Ministry of Health and B.M.A. (1934, Ref. 68) Calories (gross) *
	Utilisable Calories	Coefficient	
0–1	600	0·2	—
1–2	900	0·3	900–1,100
2–3	1,200	0·4	1,100–1,400
3–6	1,500	0·5	1,400–1,700
6–8	1,800	0·6	1,700–2,000
8–10	2,100	0·7	2,000–2,300
10–12	2,400	0·8	2,300–2,800
12–14	2,700	0·9	2,800–3,000
Boy 14–18 . . .	3,000	1·0	3,000–3,400
Girl 14–18 . . .	2,500	0·83	2,800–3,000
Man : Heavy work .	—	—	3,400–4,000
,, Moderate work .	—	—	3,000–3,400
,, Light work . .	—	—	2,600–3,000
Woman : Active work .	—	—	2,800–3,000
,, Housewife .	—	—	2,600–2,800

* Calories which food eaten could yield.

The League of Nations Report, 1936 (Ref. 69), gives figures for an ordinary everyday life without manual work in a temperate climate as follows :—

Age	Calories net *	Coefficient
1–2	840	0·35
2–3	1,000	0·42
3–5	1,200	0·5
5–7	1,440	0·6
7–9	1,680	0·7
9–11	1,920	0·8
11–12	2,160	0·9
12–15 and upwards	2,400	1·0
Man or woman	2,400	1·0
Pregnant woman	2,400	1·0
Nursing woman	3,000	1·25
Babies 0–6 months	100 Calories per kg. body weight.	
,, 6–12 ,,	90 ,, ,, ,, ,,	

Additions for muscular activity :—

Light work	Up to 75 Calories per hour of work.
Moderate work	75–150 ,, ,, ,, ,,
Hard work	150–300 ,, ,, ,, ,,
Very hard work	300 and over ,, ,, ,, ,,

Average supplement for muscular work, 600 Calories.

Housework to be reckoned as light work for eight hours per day.

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* Calories net = amount of energy available from food actually assimilated.

Values for the Calorie requirement in a large number of occupations are given in Ref. 60. The *coefficient* is the amount of food required compared to the average man as a unit. The coefficients in the first table are taken in this country as *man-values* for statistical purposes. A family consisting of man, wife, and children of eleven, seven and four years of age would have a man-value of 1 + 0·83 + 0·8 + 0·6 + 0·5 = 3·73 and would require 3·73 × 3,000 Calories per day. People over sixty-five are given a man-value of 0·75.

It must be emphasised that the foregoing values are average values and not necessarily applicable to a given individual. The dietitian is well aware that the nervous and endocrine " make-up " or habits of an individual may alter his requirements from those expected. The recommended figures are more suitable as a guide to feeding communities than individuals who have idiosyncrasies. See also U.S. Research Council recommendations p. 400.

Mental Work. There is as yet no certain evidence that mental work involves any significant increase in metabolism.

CHAPTER XXX

PRINCIPLES OF NUTRITION

(64–72, 84, 91, 95, 98–99)

In his Foreword to Friend's " Schoolboy " (Ref. 67) Drummond starts : " It is a fact at once surprising and humiliating that with thousands of years of human life and experience behind us we are actually engaged to-day in acquiring laboriously the knowledge necessary to enable us to feed and rear our children properly." We are only just beginning to realise how adults should be fed. The scientific study of nutrition in this century has revealed how complex is the problem. When it is recalled that thirty years ago the dietitian's horizon did not extend beyond Calories and digestibility, we need not be discouraged by our present incomplete knowledge of nutrition. Contrary to popular opinion, humanity has little natural instinct for correct feeding, and, in the words of Sir Frederick Hopkins,* " it would be easy to show that faulty nutrition has played a large part in inhibiting human progress, and even to show that few races have at any time been ideally nourished." We are prone to a deliberate abuse of our bodies by overeating and overdrinking ; we fall victims to scurvy and rickets without making any instinctive effort to find antiscorbutic or antirachitic foods. Some primitive tribes select a good diet, whereas others with equal natural opportunities do not. Religious vetoes and tribal customs have helped to obliterate such instincts even to the extent of causing epidemics of deficiency diseases, *e.g.*, night-blindness in religious fasts, scurvy in besieged Indian troops refusing meat. Our only common instinct is for salt. Many animals, on the other hand, have strong cravings for essential substances. Buffaloes and deer travel many miles for salt ; ruminants on phosphate-deficient pastures gnaw the bones of any carcasses they can find ; rats tend to select a properly balanced diet (Ref. 35). Drummond observed in pigs a tendency to make good a deficiency of vitamin A and of calcium.

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* *Nutr. Abst. Rev.*, **1931, 1, 3.**

Fortunately there are at the present time many active workers studying nutritional problems, so that in the near future we may hope to eliminate such penalties of dietetic ignorance, as dental caries and minor digestive troubles. A correct diet must provide for the maintenance of the body as well as energy requirements, and, where necessary, for growth and reproduction. Essential elements lost from the body by excretion must be replaced. The more important factors which need consideration are :—

- (1) Energy value.
- (2) Quality and quantity of
    - (*a*) Primary foods.
    - (*b*) Minerals.
    - (*c*) Vitamins.
- (3) Variation in the diet.
- (4) " Digestibility."
- (5) Cooking.
- (6) Psychological factors.
- (7) Cost.

(1) ENERGY VALUE

The energy requirements of an individual have been discussed in detail in Chapter XXIX. Three thousand Calories have been estimated as the daily requirement of the average " man " (see " man values," p. 384). In the past there appears to have been little undernourishment of adults except in times of stress and in institutions. In schools it was not realised that children's needs were out of proportion to their size. Accurate records are very scanty. London prisons in 1837 allowed 1,950 Calories, increased to 2,400 in 1864 ; Christ's Hospital School allotted 1,900 Calories in 1704 compared with 2,930 in 1929. Scientific study at the end of the last century focussed attention on Calories, with a resulting improvement in institutional allowances. Rowntree gave the following figures for York in 1900 :—

Families with income below 26*s*. per week	2,901	Calories.
Workhouse	3,702	,,
Prison (Class B)	3,038	,,
,, (hard labour)	4,159	,,

A recent survey of 251 families in St. Andrews, Cardiff and Reading by Cathcart and Murray (Ref. 70) averaged 3,008 Calories per "man," very few families coming below 2,500 (incomes below 8*s*. 6*d*. per head per week). Orr (Ref. 66), by dividing the total food supply by the population, gave 3,246 Calories as the average purchased per head of population in 1934 in the United Kingdom.

(2) QUALITY AND QUANTITY OF THE CONSTITUENTS OF FOOD

Whereas undernourishment is not ordinarily common in this country, malnourishment is frequent. From the meagre data available it has been surmised that apart from famine, plague and war, there was relatively little malnourishment in homes in this country (except for vitamin D deficiency), until the advent of the industrial era brought about such devastating changes in living conditions (*e.g.*, altered food supply, overcrowding and bad sanitation) that, according to Sir Arthur Keith, even the shape of the English face was changed. The crowding into towns and consequent transport and storage difficulties led to the use of white flour (p. 360) and considerable decrease in the consumption of milk, fresh vegetables and fruit. The poverty induced by the appalling labour conditions caused the replacement of butter by vitamin-deficient margarine. By 1900 the malnourishment and its concomitant diseases were rife amongst workers whose diet consisted mainly of white bread, margarine and tea and was seriously deficient in minerals and vitamins. Even in well-to-do families, butter and fresh fruit were often regarded as a luxury rather than a necessity for children. To-day, while much has been done as a result of increased knowledge, there is still a high incidence of rickets, dental caries, and dietary anæmia due partly to ignorance and partly to poverty, and not confined to town dwellers. An outstanding advance of recent times is the introduction of cheap milk for schools, following upon the scientific proof that children, given a supplement of milk (whole or separated), showed better development over a given period than on any other supplement of the same calorific value. These experiments were carried out on about 20,000 schoolchildren in Great Britain and the average growth rate was 20% greater than that of those not receiving milk as a supplement. Even greater increases have been observed in other countries in children on a more inferior basal diet.

(*a*) Primary Foods (Protein, Fat and Carbohydrate)

In this country, protein, fat and carbohydrate are consumed in the approximate ratio 1 : 1 : 4. One hundred grams each of protein and fat and 400 g. of carbohydrate would provide 3,000 Calories. The table gives figures per " man " found by Cathcart and Murray (Ref. 71) and per " head " of population by Orr (Ref. 66).

AVERAGE FOOD CONSUMPTION PER MAN PER DAY

Town	No. of Families	Protein	Fat	Carbohydrate	Calories
St. Andrews . . .	149	81	113	401	3,027
Cardiff . . .	53	77	107	436	3,096
Reading . . .	49	75	100	409	2,896
Whole population, per head (Orr) . .	—	87	124	425	3,246

Although the 1 : 1 : 4 ratio is customary in this country, wide variations, particularly of carbohydrate and fat, are possible without harm, although it is advisable that 10%–15% of the total Calories should be obtained from protein, 20%–35% from fat, and 50%–66% from carbohydrate. Some provisos, however, must be made as to the nature of the primary foods, since an arbitrary selection of certain proteins, fats and carbohydrates even in the 1 : 1 : 4 ratio might not yield an adequate diet.

Proteins.

Although the desirable amount of protein is in the region of 100 g. per day, nitrogen equilibrium and health can be maintained on considerably less. Chittenden estimated the daily nitrogen requirement of man as about 0·11 g. per kg. body weight (= 0·69 g. protein), *i.e.*, a 70 kg. man would need 50 g. of protein, and 60 g. should be ample. Experimentally it has been shown that the protein intake can be reduced to 20 g. per day, although such a figure is not suitable for general dietetic purposes. Chittenden's figure of 60 g. is satisfactory, provided the protein is carefully selected and energy is adequately supplied by fat or carbohydrate. The League of Nations Report (Ref. 69) recommends that adults should not take less than 1 g. protein per kg. body weight ; larger amounts are necessary for growth and pregnancy.

LEAGUE OF NATIONS RECOMMENDATION FOR PROTEIN REQUIREMENT

Age	Grams per kg. Body Weight per Day
1–3	3·5
3–5	3·0
5–15	2·5
15–17	2·0
17–21	1·5
Adults	1·0
Pregnant women, 0–3 months	1·0
,, ,, 4–9 ,,	1·5
Nursing women	2·0

Increased amounts would, of course, be necessary in convalescence from a wasting disease.

In the past an excessive intake of protein was thought to be dangerous. Recent experiments on animals and dietary surveys do not support this. The older experiments purporting to show ill-effects from high protein feeding were confused by lack of B vitamins and probably aggravated by infective conditions promoted by undigested protein. Recent experiments with carefully balanced diets show that animals can take 70% of their total intake as protein without ill-effects, or any perceptible strain on the kidneys. Examples of communities with a high protein intake are the Eskimos (280 g. out of 2,600 Calories) and men of the Masai, an East African tribe of good physique (300 g. out of 3,100 Calories). The objection to a large protein intake in this country is mainly an economic one, since protein is not only expensive but also has a high specific dynamic action ; neither of these factors apply in the Arctic region, since protein and fat are the most readily available foods, and the heat of specific dynamic action can be used for maintenance of body temperature.

The nutritive values of different proteins are not the same, and the quantities referred to above apply to mixed proteins, such as would be obtained in an ordinary varied diet. Some proteins, if they be the sole source of nitrogen, will not support life, whereas others are adequate. Young animals fed on gelatin as the sole source of nitrogen cease growing and die, whereas on caseinogen they flourish. Dietetically, therefore, we distinguish two types of proteins, **first-class** or **good** protein and **second-class** or **poor** protein. Proteins of the former class are capable of supporting life in the absence of other forms of protein. It is a curious but undisputed fact that proteins of animal origin (*e.g.*, meat, milk and eggs) are almost completely utilised, whereas from 10% to even 40% of vegetable proteins (*e.g.*, those of flour, peas, beans, potatoes) may remain unabsorbed and excreted in the fæces ; further, with the exception of gelatin, almost all animal proteins commonly consumed are first-class proteins, whereas vegetable proteins are frequently poor proteins. It is, therefore, more practicable to distinguish **animal** and **vegetable** protein than first- and second-class protein. In this country approximately equal amounts of each are consumed ; Orr's figures for 1934 are animal protein 46 and vegetable protein 41 g. per day per head. It is advisable to take at least 50% of total protein in the form of animal protein.

Animal proteins are usually associated with fat, but very little carbohydrate (except milk), whereas vegetable proteins are almost invariably associated with a large amount of carbohydrate. The bulkiness of vegetable foods considered as a source of proteins is illustrated by the following examples.

PERCENTAGE OF PROTEIN IN DRIED FOODSTUFFS

Animal protein	Lean beef	82
	Fat beef	51
	Egg	56
Vegetable protein	White bread	11
	Potato	10
	Butter beans	23
	Brazil nuts	20

Apart from absorbability, the difference in the nutritive value of proteins depends on their amino-acid content. One function of assimilated protein is to provide amino-acids for the building up of tissue proteins. The body appears to be able to synthesise some, but not all, so that certain amino-acids must be provided in the diet. These are called **essential amino-acids,** and are more abundant in animal proteins. The classical experiments of Osborne and Mendel on feeding rats on diets in which a purified protein was the sole source of nitrogen showed the adequacy of, amongst others, caseinogen, lactalbumin, ovalbumin, maize and wheat glutelins, and the inadequacy of gelatin, zein (maize gliadin), and several vegetable proteins.

The latter group only supports growth if certain amino-acids are added. Gelatin requires the addition of cystine, isoleucine, tryptophan and valine, and zeïn must be supplemented by lysine and tryptophan. Reference to the table on p. 102 will show that these proteins are deficient in the amino-acids mentioned. Further knowledge of essential amino-acids has been obtained by noting the effect of feeding mixtures of all the amino-acids, except the one under test. Apart from the difficulty of knowing the correct proportions of amino-acids to give, the periodic discovery of new amino-acids in proteins has entailed revision of former work, so that we do not yet know with certainty which amino-acids are essential. From recent extensive experiments on the needs of growing *rats*, Rose gives as essential and non-essential the amino-acids listed on p. 392.

Arginine can be synthesised by the rat but not fast enough for *normal* growth. Cystine is non-essential but stimulates growth when methionine is suboptimal. (Cystine and methionine must provide the bulk of the sulphur required by the animal.) Growth is satisfactory on the ten essential amino-acids alone; it is not known if other functions, *e.g.*, reproduction, are adequately provided for by these ten. It must be stressed that this information has been obtained from rats; experiments on large animals are inhibited by the difficulty of obtaining pure amino-acids in quantity. Nevertheless, it is wise to assume that these results do apply to man, since human experience has shown the nutritive superiority of animal proteins over vegetable proteins, which tend to be deficient in these essential amino-acids.

NUTRITIVE VALUE OF AMINO-ACIDS

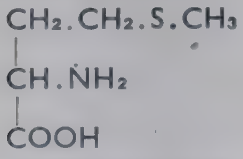
Approx. amount ‡ Necessary for Normal Growth	Essential	Non-essential
0·2	Arginine	Alanine
0·4	*Histidine	Aspartic Acid.
0·5	†Isoleucine	Citrulline
0·9	†Leucine	Cystine
1·0	†Lysine	Glutamic Acid
0·6	*Methionine	Glycine
0·7	*Phenylalanine	Hydroxyglutamic Acid
0·6	†Threonine	Hydroxyproline
0·2	*Tryptophan	Norleucine
0·7	†Valine	Proline
		Serine
		Tyrosine

* Both optical forms serve.

† Only natural form serves.

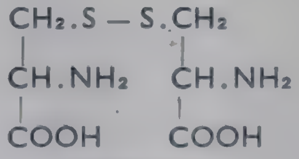
‡ As percentage of total rations, which include liberal amounts of non-essential amino-acids to cover nitrogen requirements. (Data from *Science*, 1937, 86, 298, and Ref. 63.)

These amino-acids provide another example of the extraordinarily specific nature of chemical reactions in the body. The behaviour of the optical isomers is noted in the table above. Some of these amino-acids are replaceable by the corresponding hydroxy or ketonic acids (histidine, leucine, isoleucine, phenylalanine) analogous to the inter-relation between alanine, lactic and pyruvic acids. Cystine, methionine, lysine and tryptophan, however, cannot be replaced by the corresponding hydroxy acids, although tryptophan is replaceable by indole-pyruvic acid ; valine can be replaced by its hydroxy acid. This specificity is the more curious since cystine, which cannot be formed from di-(β-thiol-lactic acid), can be formed from methionine. (Grass proteins contain methionine and very little cystine, whereas wool keratin, the crop of which is dependent on the sulphur of the pasture, contains mainly cystine.)



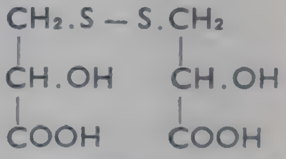
$$\begin{array}{l} \mathrm{CH_2.CH_2.S.CH_3} \\ | \\ \mathrm{CH.NH_2} \\ | \\ \mathrm{COOH} \end{array}$$

METHIONINE



$$\begin{array}{ll} \mathrm{CH_2.S} & \mathrm{-\ S.CH_2} \\ | & \quad | \\ \mathrm{CH.NH_2} & \mathrm{CH.NH_2} \\ | & | \\ \mathrm{COOH} & \mathrm{COOH} \end{array}$$

CYSTINE



$$\begin{array}{ll} \mathrm{CH_2.S} & \mathrm{-\ S.CH_2} \\ | & \quad | \\ \mathrm{CH.OH} & \mathrm{CH.OH} \\ | & | \\ \mathrm{COOH} & \mathrm{COOH} \end{array}$$

DI-(β-THIOL-LACTIC ACID)

Studies with N^{15} labelled amino-acids have shown that leucine, histidine, phenylalanine, arginine and methionine readily participate in transamination. This suggests that it is the carbon skeleton of these amino-acids which is indispensable. Lysine is exceptional for if it is deaminated it is not regenerated. As to the non-essential amino-acids there is evidence, from feeding with amino-acids labelled with deuterium attached to the carbon atoms, that they can readily be formed from other amino-acids, *e.g.*, proline and glutamic acid from ornithine, tyrosine from phenylalanine.

It is convenient to define here the term "**protective,**" frequently used in discussing nutrition. The protective substances in a diet are those which are essential for specific purposes other than energy production (water excepted), *i.e.*, first-class proteins, mineral elements and vitamins, lack of which may cause deficiency diseases.

Fats.

The average individual in temperate climates cannot take excessive quantities of fat without nausea and diarrhœa ; further, since large quantities of fat would imply a diminution of carbohydrate intake, normal fat metabolism would be impaired and ketosis would result (p. 254). Most people can utilise 150 g. per day without ill-effects. The liability to ketosis appears to be largely a matter of adaptation. Eskimos, living in a region which does not produce carbohydrate foods, have acquired a high fat tolerance and normally take more than twice as much fat as carbohydrate. (Krogh gives their daily consumption as protein 280 g., fat 135 g., carbohydrate 54 g.) They are less liable to a starvation ketosis. Very obese individuals may show a similar high tolerance. Two Americans fed for a year on a diet of 100–140 g. protein, 200–300 g. fat and 7–12 g. carbohydrate without impairing their health. Under ordinary circumstances it is usual to apply the rough rule that the amount of fat must not exceed twice the amount of carbohydrate plus half the amount of protein if ketosis is to be avoided. Man appears to have abnormally low tolerance for fat ; animals can take a considerably greater proportion.

It is usually considered that an intake of not less than 50 g. of fat per day is desirable, although this is by no means universally put into practice. In the Far East it is generally less for economic reasons ; fat only accounts for 6% of the total energy value of the diet of some Japanese. The daily intake of the East African Kikuyu tribe is protein 99 g., fat 22 g. (= 9·2% of total Calories), and carbohydrate 390 g. (Orr and Gilks). A fairly large fat intake is desirable, not because true fat is indispensable[*] to the body, but because of the valuable adherents of natural fats, notably the fat-soluble vitamins and carotene. If these adherents are supplied in other ways, experimental animals can live on the minute traces of fat which cannot easily be removed from proteins and carbohydrates. While such extremes have not been tried on man, there is evidence that he can live on diets containing very small amounts of fat.

Apart from the valuable adherents, there are two good reasons for inclusion of fat in a diet : (1) A lack of fat causes a feeling of hunger shortly after a meal, probably due to rapid digestion in its absence (the chief absorption of fat probably occurs five or six hours after a meal compared with three or four hours for carbohydrate) ; (2) In no food is the energy so concentrated as in fat ; apart from its calorific value being two and a quarter times that of protein or carbohydrate it is the only food taken practically dry, since neither proteins nor polysaccharides are palatable without water. Fat, therefore, is invaluable in reducing the bulk of the food. A moderate intake of fat is also of value in assisting the absorption of calcium and carotene.

In this country the consumption of fat is usually over 80 g. per day. It tends to increase up to about 150 g. with an increase of family income (fats are more expensive than carbohydrates). As sources of energy there is little difference between animal and vegetable fats. Animal fats are said to have a better biological value because they have more valuable adherents, vitamins A and D in particular. In this country animal fat predominates in our diet. The nature of the fats consumed in different countries largely depends on local resources. Animal fats are most used when the incidence of sunlight is low. There is probably a big individual variation in the consumption of fat, especially of meat fat, which some habitually leave at the side of the plate.

Carbohydrates.

Carbohydrate forms the greatest proportion of the diet of most races, because it is the cheapest form of food. It has the advantage that it can be readily digested, absorbed and utilised for providing energy (p. 380), but in palatable form it is bulky and liable to undergo fermentation, producing acid (*e.g.*, lactic) if digestion is delayed. It is for this reason that it is not desirable entirely to replace fat by carbohydrate. The consumption of 100 g. of fat would ordinarily entail the ingestion of about 110 g. of material (the 10 g. representing water and adhering substances); the energy equivalent of 100 g. of fat would be 227 g. of dry carbohydrate which would be obtained in 450 g. of bread, or 1,130 g. of boiled potatoes. The bulk could, of course, be reduced by taking some pure sugar, but usually only children find large amounts of this substance palatable. It is usually recommended that carbohydrate should not account for more than 66% of the total Calories—although this amount is frequently exceeded on small incomes.

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[*] It has been shown that traces of certain unsaturated fatty acids, probably linoleic or linolenic, are essential. The amounts required, however, are so small that human dietaries could not conceivably be deficient in this respect, since it is extremely difficult to remove the last traces of fat from foodstuffs.

Sufficient carbohydrate should be included in the diet to ensure complete oxidation of fat and prevent ketosis. For 100 g. protein and 150 g. fat, 50 g. carbohydrate would be adequate. As mentioned on p. 393, men have been maintained in health on a definitely ketogenic diet. Such a diet, even if dietetically permissible, is uneconomic.

As to quality, any carbohydrate which gives glucose, galactose, fructose or mannose on digestion in the animal can be utilised. In man, this means that the chief carbohydrates are starch and sugars (from milk, fruits and vegetables as well as pure sucrose); glycogen would only be consumed in living tissues such as oysters. The natural forms are to be preferred to purified forms of carbohydrate on account of valuable adherents, *e.g.*, vitamins B and E and mineral elements in cereals, vitamins B and C and minerals in potatoes, and vitamin C and minerals in fruits. Carbohydrates are almost entirely derived from vegetable sources and include cellulose which, while not appreciably digested in the human alimentary canal, is dietetically valuable since its bulk stimulates peristalsis. Cereals have recently come under suspicion as interfering with normal calcification. This is due to nearly 50% of the phosphorus being present in a non-utilisable form (phytin, p. 300). In our present state of ignorance it is probably wise to mix our carbohydrates as well as the other dietary constituents and not rely upon any one source.

Galactose can be readily synthesised in the body and there seems to be no advantage in giving extra lactose or galactose to lactating women. Pentoses and their polysaccharides are of no dietetic value. If they are absorbed they are excreted unchanged.

The consumption of refined sugar has increased enormously in recent years; whereas in 1837 it was 20 lbs. per head per annum, in 1936 it was 90 lbs. (*i.e.*, nearly 4 oz. per day). This means that about one quarter of our total carbohydrate is taken in a form free from protective adherents, a fact which detracts from the value of sugar as a food. Many hold that sugar is a contributory cause of dental caries; as yet this has been neither proved nor disproved. There is no doubt that sugar is a valuable adjunct for muscular work, especially in lessening fatigue. We have yet to discover the optimal amount of refined sugar in our diet. The present restriction of sugar consumption by rationing is probably a nutritional benefit.

(*b*) **Minerals**

Small amounts of inorganic salts are always excreted and must be replaced. Their importance, not only as tissue components but also in controlling reactions, has been explained in Chapters XXIII and XXIV. Agriculturally, pastures deficient in Ca, P, Mg, Mn and Co have caused serious diseases in grazing animals. In man, most attention has been focussed on Ca, P, Fe and I, since under ordinary circumstances deficiencies in other elements are rare. There is no uniformity of opinion as to the amounts which should be taken. Stiebeling recommends 0·9 g. Ca, 1·23 g. P and 0·013 g. Fe per head per day, based upon American dietary surveys. The findings of Cathcart and Murray per "man" and the averages from Orr's calculations per head for this country are as follows :—

INTAKE OF CA, P AND FE IN GRAMS PER DAY

	Found in Cathcart and Murray's Groups per "Man"	Calculated per Head from Orr	Stiebeling's Standard (per head)
Calcium . . .	0·63–1·38	0·66	0·9
Phosphorus . .	1·24–2·19	1·22	1·23
Iron . . .	0·012–0·029	0·011	0·013

The figures are essentially in agreement since per head values would be lower than per " man " values. The values cannot, however, be regarded in the same light as those for the primary foodstuffs where requirement can be measured ; we have no means of assessing our needs of mineral elements. The Stiebeling figures correspond to those consumed by people whose diet we believe to be adequate in every way. On the basis of the amount excreted 0·6 g. of Ca should be sufficient.

Calcium. Increased amounts of Ca are needed by children, pregnant and nursing women. Stiebeling recommends at least 1 g. per day for children compared to 0·68 for an adult man, and the League of Nations Report 1·6 g. per day for the pregnant or nursing woman. The best sources are cheese and milk in which calcium is in a readily absorbable form. Other sources are eggs, green vegetables, oranges, butter-milk, nuts, beans and carrots. Hard water can be regarded as a source of calcium and may provide 5% or 10% of the total. Salts, such as the carbonate, phosphate and lactate, can be utilised. Meat, fish, white bread and fruits are poor sources.

Phosphorus. This element is taken either as organic or inorganic phosphate. Children should have an intake of 1 g. P per day, adults 1·3 g., and a pregnant or nursing woman 1·9 g. The best sources are animal foods, such as meat, fish, milk, cheese and eggs ; of the vegetable foods beans, lentils, rye, oatmeal, and wholemeal flour provide a considerable proportion of the total phosphorus. The latter contain part of their phosphorus in the form of phytic acid, which not only cannot be utilised but hinders absorption of calcium, so that if additional phosphorus is needed it is better to use animal sources.

Iron. Although the amount taken is small, iron is an important constituent of the diet, and a deficiency leads to a form of anæmia. It is usually considered that there is little risk of a deficiency in an ordinary mixed diet,* although anæmia, in some degree, is common in women and children in the lower income groups ; this cannot, however, be ascribed solely to iron deficiency. The bulk of the iron in a mixed diet is from bread, meat and potatoes. Good sources are liver, kidney, egg-yolk, green peas, spinach, watercress, cabbage, potatoes, carrots and cereals. Not all forms of iron are readily utilised, especially the non-ionisable forms; only about a quarter of the iron of meat can be taken as available, since hæmoglobin is poorly utilised. Milk is poor in iron. The infant is born with a store of iron which, with that of the milk, usually suffices for about six months; after this period supplements of egg-yolk, purées of green vegetables or carrots are usually recommended as a safeguard against anæmia. Human milk contains 1–2 mg. iron per litre, cow's milk less.

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* A recent study, however, of the intake of *available* iron in sixty-three middle-class homes by Widdowson and McCance indicated a suboptimal intake by the women (8 mg. p.d.), but not by the men (11 mg. p.d.). Only the women's hæmoglobin level was raised by giving additional iron.

Iodine. Normally the minute amounts of iodine which are essential (0·05 mg. per day) are obtained from water, vegetables and fish. The iodine of the plants is derived from the soil and water. In certain localities where the soil and water lack this element, dietary deficiency may cause simple colloid goitre (p. 337); apart from these localities there seems no need to consider the iodine content of the diet. Fish, cod-liver oil and vegetables which have been grown in iodine-containing soils can be used to supplement iodine-deficient diets. In the localities where iodine is lacking in the soil and water, inorganic iodides may be added to the salt taken in the diet.

Sodium Chloride. This salt is ordinarily consumed in amounts (about 20 g. per day) greatly in excess of requirement. There is ample in ordinary food without the addition of salt as a condiment. Sodium chloride intake only needs consideration if abnormal quantities are lost in sweat during severe muscular exercise at high temperatures. Under these circumstances ingestion of large quantities of water to relieve thirst is liable to produce a serious condition known as "*miners'*" or "*stokers'*" *cramp.* The condition is prevented by taking salt solutions instead of water.

Magnesium. There is no clinical abnormality by which a deficiency of magnesium can be assessed. Analyses of American dietaries indicate an average daily consumption of about 0·2 g., but it is not known whether this amount is adequate or suboptimal. Good sources of magnesium are green vegetables, meat and bread.

Copper. Since all foods contain copper in some degree there is little risk of a deficiency. A daily adult requirement of 2 mg. has been suggested. Good sources of copper are liver, oysters, cocoa, nuts, apricots, currants and lettuce. Human and cow's milk only contains about 0·6 mg. per litre.

Acidity and Alkalinity of Foods. It is sometimes necessary to adjust a diet on account of a wrong acid-base balance. Since foods are ultimately completely oxidised in the body, we can assess their acidity or alkalinity by examining the ash left on incineration. Some typical examples are :—

REACTION OF ASH OF FOODSTUFFS

Acid	Neutral	Alkaline
Cereals	Sugar	Milk
Eggs	Animal and vegetable fats	Blood
Meat		Peas
Fish		Beans
Cheese		Most roots and tubers
		Most fruit juices

(*c*) Vitamins

The importance and sources of the vitamins are recorded in Chapter XXVIII. The recommended daily requirements are given in the following table. The International units adopted by the League of Nations are defined on p. 436.

This section is appropriately concluded by quoting (on the next page) the daily requirements of the various nutrients recommended by the U.S.A. National Research Council's Committee on Foods and Nutrition.

(3) VARIATION IN THE DIET

A varied diet tends to eliminate the risk of missing some essential element or vitamin, and is of value psychologically in making food more palatable. The varied diet of Europeans and Americans is by no means universal. Many communities are limited in their choice by environment ; Eskimos live largely on fish and meat, whereas the poorer Orientals exist mainly on rice with small amounts of fish. Some people live by choice (or laziness ?) on diets which most would consider very monotonous.

(4) " DIGESTIBILITY " OF THE FOOD

A factor of considerable importance in dietetics is the " digestibility " of the food. A protein which contains all the essential amino-acids will be dietetically valueless if it is not

RECOMMENDED DAILY ALLOWANCES

(Committee on Foods and Nutrition, National Research Council, U.S.A.)

	Calories	Protein, Gm.	Calcium, Gm.	Iron, Mg.	Vitamin A, International Units	Vitamin B_1, Mg.	Riboflavin, Mg.	Nicotinic Acid, Mg.	Ascorbic Acid, Mg.	Vitamin D, International Units
Man (70 Kg.)										
Moderately Active	3,000	70	0·8	12	5,000	1·8	2·7	18	75	
Very Active	4,500	—	—	—	—	2·3	3·3	23	—	
Sedentary	2,500	—	—	—	—	1·5	2·2	15	—	
Woman (56 Kg.)										
Moderately Active	2,500	60	0·8	12	5,000	1·5	2·2	15	70	
Very Active	3,000	—	—	—	—	1·8	2·7	18	—	
Sedentary	2,100	—	—	—	—	1·2	1·8	12	—	
Pregnancy (latter half	2,500	85	1·5	15	6,000	1·8	2·5	18	100	400–800
Lactation	3,000	100	2·0	15	8,000	2·3	3·0	23	150	400–800
Children up to 12 years:										
Under 1 year	100/Kg.	3–4/Kg.	1·0	6	1,500	0·4	0·6	4	30	400–800
1–3 years	1,200	40	1·0	7	2,000	0·6	0·9	6	35	—
4–6 years	1,600	50	1·0	8	2,500	0·8	0·9	8	50	—
7–9 years	2,000	70	1·0	10	3,500	1·0	1·5	10	60	—
10–12 years	2,500	70	1·0	12	4,500	1·2	1·8	12	75	—
Children over 12 years:										
Girls, 13–15 years	2,800	80	1·3	15	5,000	1·4	2·0	14	80	
,, 16–20 years	2,400	75	1·0	15	5,000	1·2	1·8	12	80	
Boys, 13–15 years	3,200	85	1·4	15	5,000	1·6	2·4	16	90	
,, 16–20 years	3,800	100	1·4	15	6,000	2·0	3·0	20	100	

The allowances recommended above should be sufficient to maintain complete dietary health. Allowances for children are the needs for the middle year in each group. The figures for vitamin A could be less if it is provided as preformed vitamin A. Vitamin D is required by older children and adults. When none is available from sunshine amounts up to 400 I.U. may be required. The actual need for B vitamins will depend upon an individual's ability to synthesise them (p. 350) broken down in the alimentary canal. The primary foods must be of such a nature that they can be both digested and absorbed. In its dietetic application "digestibility" is more concerned with absorbability than digestion in the strict biochemical sense; figures for the time which a food stays in the stomach are of less interest than the amount which is finally absorbed and metabolised. A complicating factor is dietetic habit. To a European the diet of the Eskimo would be "indigestible," at any rate at first; many English people cannot suddenly adopt even the normal German diet without evidence of "indigestion" which later disappears as they become accustomed to the new diet.

For absorbability, useful figures can be obtained by measuring the proportions of a given food which are excreted in the fæces. This reveals that vegetable proteins are not absorbed as well as animal proteins, while fats and starch are generally largely utilised. The following figures are given by Hutchison and Mottram (Ref. 65):—

Food		Percentage not Absorbed		
		Protein *	Carbohydrate	Fat
Animal	Milk	7·1	—	5·3
	Beef	2·6	—	—
	Eggs	2·6	—	5·0
Vegetable	Bread	21·8	1·1	—
	Peas	17·5	3·6	—
	Potatoes	30·5	7·4	—

* The protein figures (calculated from the fæcal nitrogen) are somewhat exaggerated, especially in the foods with low proportions of protein, *i.e.*, the vegetable foods, since a considerable part of the normal fæcal nitrogen is independent of the food nitrogen.

Absorption is usually better with a mixed diet than when one substance is taken alone. It is usually taken that from 78% to 91% of protein, 86% to 95% of fat and 93% to 97% of carbohydrate will be absorbed.

(5) COOKING (30)

Foods undergo considerable change in the processes of cooking and preparation. Inedible portions are removed and harmful organisms are destroyed. The chief difference between raw and cooked meat is that the latter, even boiled, has less water, so that 4 g. of cooked meat have the nutritive value of approximately 5 g. of raw ; the soluble protein is, of course, coagulated. Some fat and extractives are lost, and collagen fibres are converted into gelatin, thus loosening the muscle fibres. Cooking does not necessarily increase the digestibility—clinical practice, in fact, suggests that well-disintegrated raw or underdone meat is the most easily digested. But cooking, by breaking down connective fibres, makes meat easier to masticate and so assists digestion ; to this must be added the greater palatability of cooked meats. Overcooking, by causing shrinkage of coagulated protein, decreases the digestibility. Cooking usually increases both the water content and digestibility of vegetables ; here the chief effect is the loosening of the cellulose framework and the liberation of the starch from starch grains (raw starch is practically indigestible). Fats are little changed in cooking. Cooking enhances the flavour of food by the addition of seasoning, and, in dry cooking (roasting, baking), by the formation of caramel from sugar and of partial decomposition products from fat and protein. Many of these substances stimulate the secretion of digestive juices.

Cooking involves loss in nearly all foods, especially of soluble substances in boiling processes. Vitamins B_1 and C are especially liable to destruction when vegetables are cooked (see pp. 360, 367, 440). The practice of making soups from the liquids in which meat and vegetables are boiled conserves soluble constituents such as salts and sugars which would otherwise be wasted. It is usually taken that 10% of the energy value of purchased food is lost in its preparation for the table, but recent surveys (*e.g.*, Ref. 71) suggest that the actual waste is below 5%.

(6) PSYCHOLOGICAL FACTORS

There is no doubt that the consumption of food may be affected by a number of psychological factors. Worry and anxiety diminish the appetite and may even upset digestion through imperfect mastication and secretion of digestive juices. On the other hand, enjoyment and consumption of food can be increased by making the food appear and smell attractive, eating it in pleasant surroundings and congenial company, and ensuring plenty of variety. These factors may assume special importance in illness and convalescence.

(7) COST (66)

Family income has a big influence on dietary. Orr (Ref. 66) in 1936 surveyed the dietaries of different income groups in this country. The population was divided into six groups as follows :—

Group	Weekly Income per Head	Estimated Weekly Expenditure on Food	Percentage of Population
I . .	Up to 10*s.*	4*s.*	10
II . .	10*s.* to 15*s.*	6*s.*	20
III . .	15*s.* to 20*s.*	8*s.*	20
IV . .	20*s.* to 30*s.*	10*s.*	20
V . .	30*s.* to 45*s.*	12*s.*	20
VI . .	Over 45*s.*	14*s.*	10
Average .	30*s.*	9*s.*	

The consumption of foodstuffs in the different groups fell into three classes which are represented by the curves * in Fig. 29.

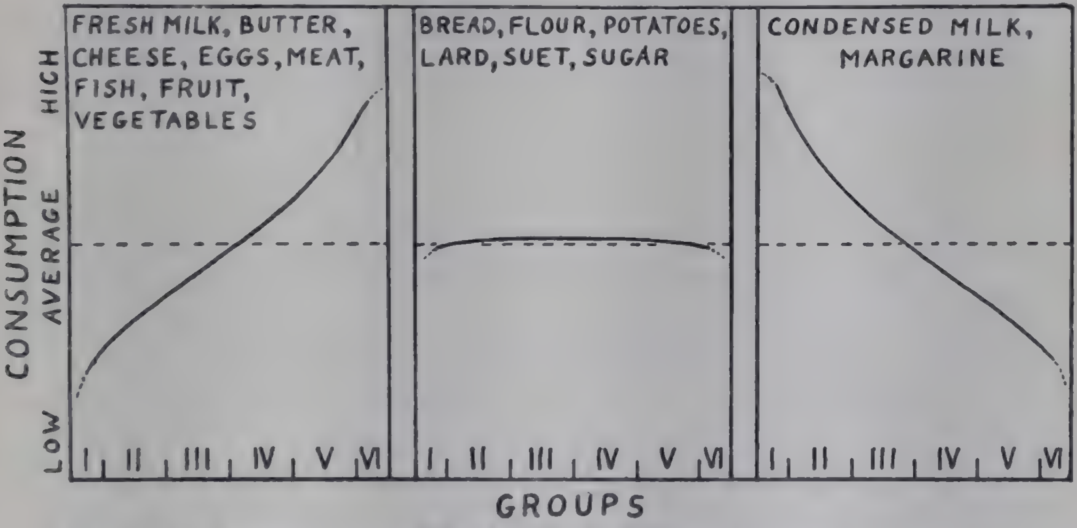

FIG. 29. Consumption of foodstuffs in different income groups. (From Orr's data, Ref. 66.)

All the protective foods fall into the class in which consumption was only high when the income was good ; a poor income meant a poor share of protective foods. The consumption of non-protective energy-giving foods was fairly uniform ; condensed milk and margarine were the privilege of poverty.

Taking as a standard a diet judged to be capable of maintaining an individual in a state of health such as cannot be improved by diet, the adequacy of the nutrition of the six groups was plotted in the form of the curves shown in Figs. 30 and 31.

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* For the curves for each food mentioned see Ref. 66.

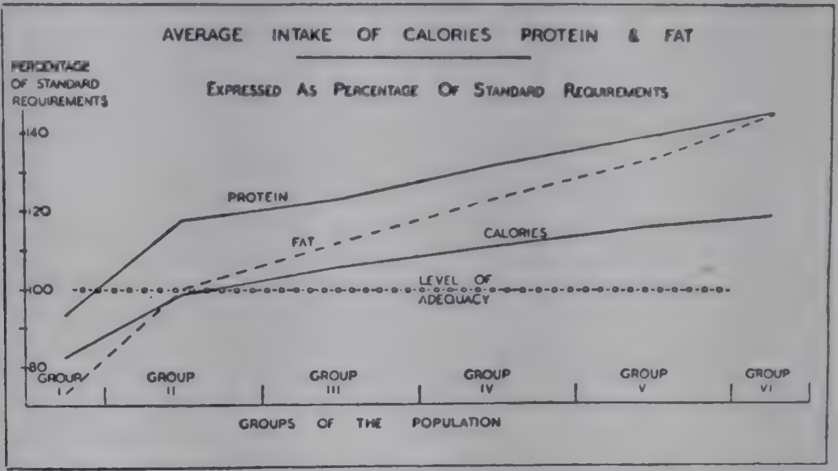

Fig. 30. From Sir John Orr, "Food, Health and Income" (Macmillan), 1936.

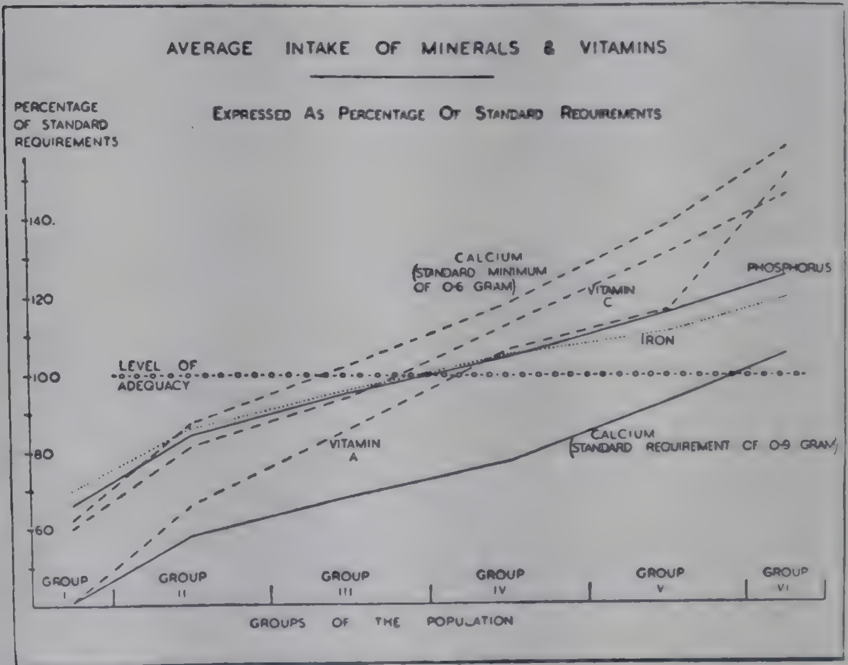

Fig. 31. From Sir John Orr, "Food, Health and Income" (Macmillan), 1936.

It will be seen that approximately half the population could be regarded as well fed. This does not mean that the remainder were all badly fed. The estimate of adequacy is probably high and malnourishment mild except in Group I; but the health of

Groups II and III could be improved by inclusion of more protective foods in their diet, although they were not in a state of ill-health which would merit the term disease. Group I were definitely malnourished, some possibly undernourished. It is desirable that all should be raised to the level of Group IV ; to do so would require an increased intake of from 12% to 25% in the more expensive protective foods, *i.e.*, milk, eggs, butter, fruit, meat and vegetables. There is little doubt that such improvements would mean a lowering in the incidence of disease and of the death rate, as well as better general physique and stature. The effect of a milk supplement on elementary schoolchildren (p. 387) showed how such a change was needed ; these children would come from homes represented by Groups I to IV. Records of height and weight from large groups of schoolboys indicated a difference in average stature proportional to the income group, *e.g.*, the average height of boys from Christ's Hospital School (Groups III–VI) was 2½ in. greater than those of Council School boys (Groups I–IV) of the same age.* As to diseases, the incidence of rickets and nutritional anæmia was highest in the lowest income groups ; this is only to be expected, since these diseases are attributed to a lack of minerals and vitamins which are only abundant in the more expensive foods. The lower groups were undoubtedly less resistant to infectious diseases such as whooping-cough, measles, diphtheria and tuberculosis ; this resistance can be raised by improved nutrition, although other factors such as housing are of importance. The effect of nutrition on susceptibility to tuberculosis is instanced by the big increase in tuberculosis mortality in industrial areas (*i.e.*, where food shortage was greatest) in Germany during the 1914–1918 war. Probably the best natural evidence of the value of good nutrition is the comparison (Orr and Gilks) of two East African tribes living under the same climate and conditions of housing. The Masai live on a diet rich in animal protein, minerals, and vitamins, whereas the Kikuyu are overloaded with carbohydrate, and lack protective foods. The average male of the former tribe is 5 in. taller than the male of the latter ; disease is twice as prevalent in the Kikuyu tribe, whose diet roughly corresponds to those of Groups I and II of our population.

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* It must be realised that we are considering the average of very large groups and that our conclusions may not apply to a specified individual. No one can overstep the limits of his heredity by altering his diet.

Relative Costs of Foodstuffs (See p. 407)

	Cost per 1,000 Calories Eaten	Price as Purchased, 1946	Protective Factors
PROTECTIVE FOODS			
Milk	1s. 0d.	4½d. pint	Good Protein, Minerals, Vitamins.
* Cheese (Cheddar) .	7d.	1s. 1d. lb.	
* Eggs	2s. 0d.	2d. each	
* Liver (ox, fried). .	1s. 9d.	1s. 6d. lb.	
* Herrings (fried) . .	7½d.	7d. lb.	
* Butter	6d.	1s. 8d. lb.	Vitamins A and D
Apples	5s. 6d.	1s. lb.	Minerals, Vitamins.
Bananas (3½ oz.) .	5s. 0d.	3d. each	
Oranges (5 oz.) . .	5s. 1d.	2½d. each	
Plums	4s. 3d.	6d. lb.	
Brussels sprouts (boiled)	7s. 0d.	6d. lb.	
Cabbage (boiled) .	11s. 11d.	4d. lb.	
Carrots (boiled) . .	3s. 2d.	3d. lb.	
Lettuce	27s. 9d.	8d. lb.	
LESS PROTECTIVE FOODS			
Mutton (boiled leg) .	2s. 1d.	1s. 6d. lb.	Good Protein.
,, (roast leg) .	2s. 4d.	1s. 6d. lb.	
,, (stewed scrag and neck) .	1s. 4d.	11d. lb.	
Beef (stewed steak) .	2s. 6d.	1s. 4d. lb.	
* Bacon (fried, streaky).	1s. 8d.	1s. 8d. lb.	
Cod (steamed) . .	4s. 5d.	1s. lb.	
Potatoes (boiled) .	5d.	1½d. lb.	Vitamins. Minerals.
* Bread (National), 85%	2d.	2½d. lb.	
* Flour (National), 85%	1½d.	2½d. lb.	
NON-PROTECTIVE ENERGY FOODS			
* Oatmeal . . .	1½d.	3½d. lb.	
* Rice (1944) . .	5½d.	9d. lb.	
* Margarine . .	3d.	9d. lb.	
* Sugar . . .	2½d.	4d. lb.	
* Butter beans . .	6d.	8d. lb.	
* Lentils . . .	3d.	5d. lb.	

* = foods of high energy value. (Over 900 Calories eaten per pound purchased.)

One disease, supposed to be largely dietetic in origin, cannot be ascribed to poverty. Dental caries is so widespread in all groups that its incidence, if it is caused by diet, must be ascribed to ignorance, although lack of protective foods may increase its severity.

It is unfortunate that the protective foods which would lower the incidence of these diseases are the most expensive and are regarded by the poor as luxuries, since they do not satisfy hunger as well as the cheaper energy-giving non-protective foods. This is clearly shown in the table on p. 406.

In calculating the Calorie costs, allowance has been made for the losses involved in preparation of the food for consumption (data from Ref. 76). The actual prices used for the calculations are given, since many of the values are liable to fluctuation. They were retail prices for cheaper quality foods, 1946. Note the high cost of fruits and vegetables and low cost of the non-protective energy foods. The cheapest protective foods are butter, cheese, herrings, milk and National bread; their deficiency in vitamin C could be made up by liberal consumption of potatoes. Note that prior to 1940 bread was white (70% extraction) and non-protective.

An experiment in Norwegian schools started in 1932 illustrates the nutritive importance of protective foods. The usual mid-day meal of hot meat and cooked vegetables, which contains relatively little protective substances, was replaced by a highly protective meal consisting of a wholemeal bread roll, butter, cheese, milk and half an apple or orange (or lettuce or raw carrot according to the season) per child. After this had been consumed bread and margarine was given to satisfy any remaining hunger. This type of meal, which aimed at making up for the lack of protective substances in the children's home diets, caused so great an improvement in general health and development that it was widely adopted in Norway.

NUTRITION IN WARTIME

No account of the nutrition of this country would be complete without reference to the war years 1939–1945. The war afforded an opportunity of subjecting our nutritional theories to a severe test with millions of men, women and children as the "experimental animals." At the same time it largely solved the problem of improving the dietary of people of the lower income groups so clearly indicated by Orr's survey of 1936. The contrast in the feeding of the people in the 1914–1918 war (see Refs. 64, 84) and in the 1939–1945 war is, indeed, a most gratifying one, for the scientifically guided policy of food control during the six years meant that the population as a whole had almost never been better fed.

Rationing and price control were immediately adopted (in the previous war full rationing was only introduced in July, 1918); unemployment was reduced to a negligible, very low level; wages were higher. These, with the other measures introduced, meant an increased consumption by the working classes of milk (20%), potatoes and vegetables (25%), calcium (25%) and vitamin B_1 (40%). Bread, fortunately, was never rationed and nutritionally it was greatly improved by compulsory raising of the percentage extraction of the flour. Whereas previously white flour of about 70% extraction (p. 424) and devoid of vitamin B_1 was generally used, the bread of the war years was made from flour of 85% extraction which retained almost all of the B vitamins originally

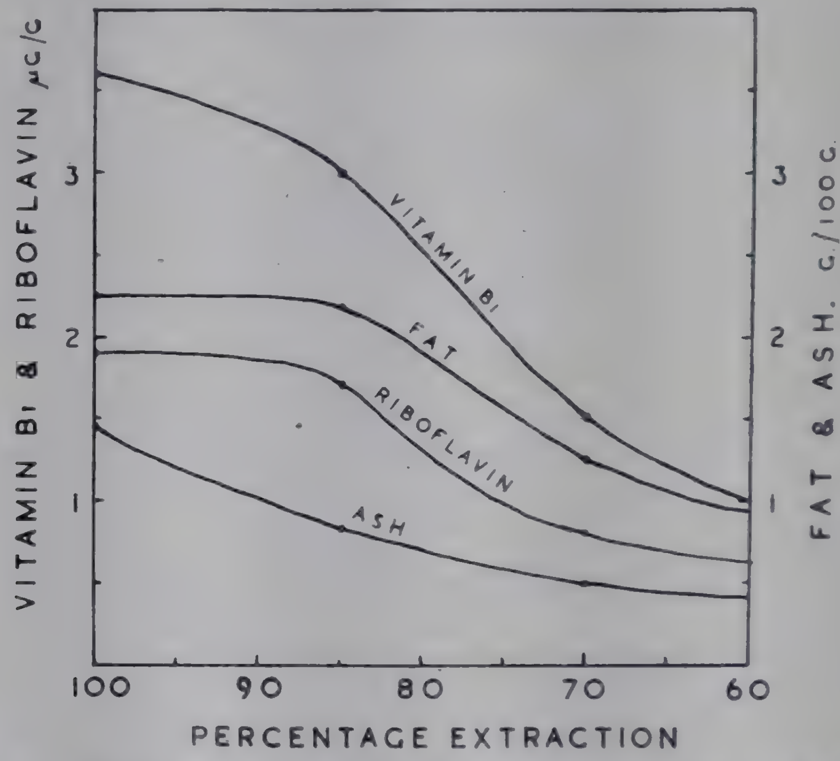

FIG. 32. Effect of milling upon the content of vitamin B_1, riboflavin, fat and minerals in wheat.

(Data from McCance *et al.*, Biochem. J., 1945, **39,** 213.

present in the wheat. The relation between the vitamin B content and percentage extraction is illustrated in Fig. 32, which shows how 85% extraction retains in the flour almost all the vitamins of the grain. Note the very sudden fall just below 85% ! Fig. 32, also shows the effect of milling upon the content of fat and minerals (as ash).

With regard to vitamins, all margarine sold for domestic use was reinforced with vitamins A and D, rendering it equivalent nutritionally to good butter. This and carrots provided the bulk of our vitamin A. Our main source of B vitamins was bread (which supplied about half), bacon and potatoes. For vitamin C we relied very largely on potatoes and green vegetables which gave about 95% of the total. For children extra vitamin C was available in the form of blackcurrant, rose-hip and orange juice concentrates. Of the mineral elements, calcium and iron were secured in bread, the former by addition of calcium carbonate to the flour and the latter as a result of the increased extraction. Calculations by the Ministry of Health in 1942 showed that if an individual consumed his rations, spent his points wisely and used available foods, he had no difficulty in securing sufficient nutrients* to satisfy the League of Nations standards. (Details of these calculations were given in the third edition of this book.)

Further measures of benefit included allowances for special needs, *e.g.*, extra cheese for farm labourers, milk and egg priority for expectant and nursing mothers and children under five years. School children were considered by provision of cheap milk and meals in school, and workers generally by cheap meals in British Restaurants and works canteens. During the period great efforts were made to educate the public about diet and the best use of available foods, so that many became food-conscious, in the nutritionist's sense of the word, and eager for scientific advice.

There is no doubt that the soundness of our nutritional theories has been proved in practice, for in spite of the restrictions and strain of the war period the general health of the population, and

* Experiments were carried out in 1939 in which volunteers lived for some months on diets more drastically restricted than those actually experienced by the population of this country at any period during the war. The experimental subjects at the end of three months of this dietary were very fit and capable of performing severe exercise tests without undue fatigue. Unfortunately the imposition of censorship prevented the publication of these interesting experiments (McCance and Widdowson, Ref. 98) until after this edition had gone to press.

of the children in particular, has been maintained, if not improved.

The two quotations from the League of Nations Report, 1937 (Ref. 72, pp. 32, 33) which have concluded this chapter in previous editions are still pertinent. Let us hope that the time may soon come when they can be discarded as out of date.

" We have argued that food habits, at least in many parts of Western communities, have gradually been tending to change in the right direction ; and have adduced statistical evidence to show that these communities, on the whole, are now consuming, in addition to the indispensable foods of high energy value, more milk and dairy products, more fruit and more vegetables than a generation ago."

" The movement towards better nutrition has made considerable progress, but it has not gone nearly far enough. Poverty and ignorance remain formidable obstacles to progress ; the disparity between food prices and incomes increases the difficulty experienced by the poorer sections of the community in obtaining an adequate supply of the proper foods."

A detailed historical account of English diet from medieval times up to 1939 is given in Ref. 84.

CHAPTER XXXI

THE NATURE AND COMPOSITION OF COMMON FOODS (65, 75, 76, 78, 79, 99)

It follows from the previous chapter that a knowledge of the proportions of protein, fat, carbohydrate, minerals and vitamins in foods is as important as their Calorie values. Tables are available (Refs. 75, 76), giving all these values except those for vitamins, and are used for the planning of special dietaries.* Comprehensive quantitative values for vitamins are not yet generally available; in many foods, *e.g.*, butter and milk, the wide fluctuations in vitamin content would render the values of little practical use. At the moment it is of more dietetic value to know of rich sources which can be used when a deficiency is suspected; these have been indicated in Chapter XXVIII. Tables of some quantitative values for vitamins are given on pp. 437–439. Mineral analyses are of restricted value because we know neither our requirement nor the percentage which we can absorb from a given foodstuff. Since, in practice, quantitative mineral analyses are mostly used for planning diets suitable for the treatment or diagnosis of certain conditions, only values for protein, fat and carbohydrate are given in the tables in this chapter.

Foodstuffs can be divided into groups of fairly characteristic chemical composition dependent upon their origin. The main divisions are: (*a*) foods from animal sources which are almost devoid of carbohydrate (except milk), and (*b*) the carbohydrate-rich vegetable foods. Animal foods are conveniently subdivided into dairy products and flesh foods.

DAIRY PRODUCTS

MILK

The value of milk has been recognised from the earliest times, and is the nearest approach we have to a perfect and complete food. It contains not only protein, fat and carbohydrate, but also valuable mineral elements and vitamins.

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* The manner in which these tables are used for making up menus is beyond the scope of this book. See text-books of dietetics, such as Ref. 65 or 73.

The chief proteins, **caseinogen** and **lactalbumin**, are both good proteins. The former is a phosphoprotein (0·7% P) and yields phosphoric acid on hydrolysis. In milk it is associated with calcium, probably as calcium caseinogenate. Caseinogen is insoluble at its isoelectric point (pH 4·6) ; this is why milk curdles on mixing with acid fruit juices. Caseinogen is soluble in neutral or alkaline solution and is not coagulated on boiling. In the form in which it occurs in milk, the solution is opalescent and contributes to the white opacity of milk. Lactalbumin is a typical albumin. Small amounts of other proteins are present.

Milk **fat** is in the form of a very fine and stable emulsion, and is the most palatable and digestible fat known. It differs from other fats in containing all saturated "even carbon" fatty acids from butyric (C_4) to lignoceric (C_{24}), as well as a variety of unsaturated acids. It has a greater proportion of fatty acids of low molecular weight than has any other natural fat.

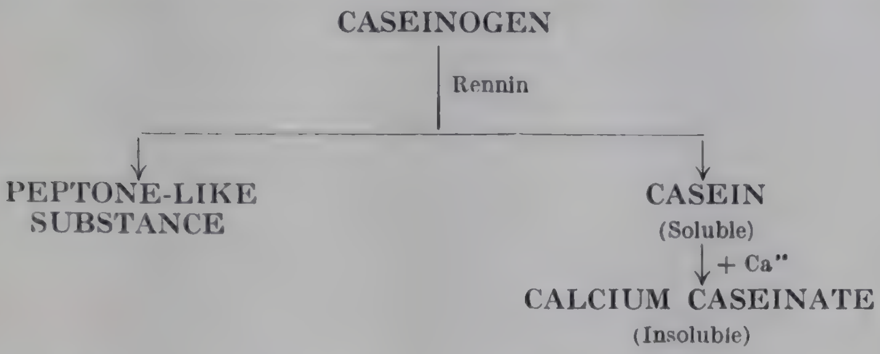
The **carbohydrate** of milk is the sugar lactose, which has a less sweet taste than cane sugar (p. 433).

Milk is rich in **mineral elements**, especially calcium, potassium, sodium, chlorine and phosphorus. The calcium is present in a form which is especially easy to assimilate. The iron content of milk is, however, low and insufficient for the suckling, which is protected by being born with a store of iron.

As a source of **vitamins**, milk is rich in A (or carotene) and riboflavin, good in B_1 and P-P factor, but poor in C and D, although the actual amounts are dependent on the mother's diet. In cow's milk there is little D in winter unless care has been taken to supplement the diet of the cow.

Cow's milk when fresh is just acid (pH 6·7) and has a specific gravity of about 1·030. On standing, unsterilised milk goes **sour**, becoming more acid, due to the formation of lactic acid from lactose by bacteria always present in milk (*e.g.*, *B. acidi lactici*); the amount of acid formed may be sufficient to precipitate (**curdle**) the caseinogen. Fresh milk does not coagulate on boiling ; but milk which is sour or acidified in some other way does. Definite changes occur in milk on boiling, as evidenced by its taste and smell ; hydrogen sulphide is said to be liberated. The skin which forms on the exposed surface of hot milk consists of calcium caseinogenate, partly coagulated lactalbumin, and fat ; if the skin is removed, a new one forms on the exposed surface. The process goes on indefinitely so long as a fresh surface is exposed to evaporation, and is not peculiar to milk, since it is seen when any protein solution mixed with fat is heated.

When milk comes into contact with rennin or proteolytic enzymes it undergoes a change known as **clotting** or **coagulation.** The exact nature of the change is not certain, but it seems that caseinogen is split by the enzyme into a soluble casein * and a soluble peptone-like substance. The clot is essentially the insoluble calcium salt of casein. The change can be represented :—



When this change takes place in milk, a jelly first forms (**junket**) ; later the clot shrinks and expresses a clear yellowish liquid called **whey** ; the fat remains entangled in the clot or **curd.** In the stomach the physical nature of the curd varies with the milk, the way it is taken and the acidity of the stomach ; it may be soft and loose, or shrunken to a tough curd which is not easily digested. Tough curds are formed in the adult stomach when milk is taken raw ; more digestible curds are formed if the milk is diluted either with fluids (*e.g.*, milk and soda) or solid foods. The proteins of milk apparently neutralise gastric HCl sufficiently to allow a clotting rather than a curdling, *i.e.*, acid precipitation, to take place.

Clotting must be distinguished from curdling. Clotting is due to enzyme action ; its product, a curd, is calcium caseinate entangled with fat. Curdling is precipitation of unaltered caseinogen by acid ; fat may be entangled in the precipitate. Junket is formed by clotting ;

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* In American and German literature, caseinogen is called casein and the English casein, paracasein, *i.e.*, in these countries the clot of milk is calcium paracaseinate.

sour milk curdles. It is unfortunate that long usage (*e.g.*, curds and whey) has established curd as the name of the product of the clotting process.

Sterilisation of Milk. The high nutritive value of milk makes it an ideal medium for the growth of bacteria, so that it is practically impossible to market raw milk in sterile condition. While milk may become a source of danger from bacteria of diphtheria, typhoid fever, scarlatina, etc., introduced during handling, the commonest danger is from the tubercle bacillus derived from the cow. The bacteria which normally cause souring are probably harmless. To combat the danger of tuberculosis, milk may be collected under hygienic conditions from certified tubercle-free herds—**tuberculin-tested milk.** All bacteria can be destroyed by keeping the milk at 110° C. for some time ; milk so treated is known as **sterilised milk.** It keeps for a long time, provided that it is not reinfected, but its taste is so altered that many find it unpalatable. Other disadvantages are that the lactalbumin is coagulated, some calcium is rendered insoluble, the caseinogen is not so readily digested, and vitamin C and the fine emulsification of fat are destroyed.

These disadvantages are eliminated by **pasteurisation**, by which the tubercle bacillus and other pathogenic bacteria are destroyed, although the souring bacteria may survive. The process consists in heating the milk rapidly to 62·5° C. (145° F.), keeping it at this temperature for half an hour and then quickly cooling. Pasteurisation, if properly conducted on fresh milk, causes no significant loss of nutritive value. There is about 20% loss of vitamin B_1 and none of riboflavin. There is little destruction of vitamin C if the milk has not been exposed to light * before pasteurisation in an apparatus in which copper does not come into contact with the milk.

A delicate test which reveals the efficiency of pasteurisation, the **phosphatase test,** consists in testing the milk for the presence of phosphatase. This enzyme, always found in raw milk, happens to be completely destroyed by the conditions of pasteurisation laid down by law. If the heating has been conducted even 1·5° F. below the specified temperature or the time of heating has been reduced by ten minutes, detectable amounts of phosphatase are left in the milk.

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* Ascorbic acid is rapidly converted into dehydroascorbic acid if milk is exposed to bright light, and the latter is rapidly destroyed by heat, especially if copper is present.

In the home, the pathogenic bacteria in raw milk can be destroyed by boiling the milk for five minutes.

Composition of Milks. The quantitative composition of milk is variable even for a given species. That of cow's milk depends on the breed and age of the cow and the time which has elapsed since calving, as well as on the nature of the food ; the milk taken at the beginning of a milking differs slightly from that taken at the end. Under present methods of milk distribution the milk of the whole herd is mixed, so that milk as usually purchased tends to have a composition within the limits :—

Water	87%–88%	Total solids 12%–13%.
Protein	3%–3·5%	
Fat	3·5%–4·5%	
Carbohydrate	4·5%–5·0%	
Ash	0·7%	

It is illegal to sell cow's milk with a fat content below 3%.

The milks of different species show great quantitative variation, especially in protein and minerals, the amounts of which are roughly related to the rate of growth of the animal for which the milk is intended. The newborn child doubles its weight in about 180 days compared with six days for a rabbit. The protein and calcium contents of the milks are 1·0% and 0·04% for human, 10·4% and 0·89% for rabbit's. The approximate composition of milks which are used for human consumption are :—

PERCENTAGE COMPOSITION OF MILK OF DIFFERENT SPECIES

	Protein	Fat	Carbohydrate	Ash
Woman	1·0	2·9	6·7	0·2
Ass	1·9	1·4	6·3	0·4
Mare	2·6	1·6	6·1	0·5
Cow	3·0	3·6	4·8	0·7
Goat	4·3	4·8	4·7	0·8

In the event of failure of the mother's milk, a baby has to be fed upon one of the other milks in the table or preparations of similar composition. Efforts are often made to adjust the new milk so as to resemble human milk. Cow's milk, for example, would be diluted until the protein concentration is correct, and cream and lactose added in appropriate amounts ; an iron salt should be added, since even undiluted cow's milk has less iron than has human milk. The final mixture is described as **humanised** milk. The preparation of a truly humanised milk is, however, an impossibility, because the constituents of milks differ qualitatively as well as quantitatively.

Human milk fat contains more oleic acid than does the fat of cow's milk, and is present in a finer state of emulsification. The phosphorus is differently distributed, being almost entirely in organic combination in human milk. The greatest difference is in the nitrogen distribution. Some recent analyses by Plimmer and Lowndes gave the following results in grams per 100 c.c. milk.

	Cow		Human	
Caseinogen . .	2·28	2·99	0·32	1·0
Lactalbumin . .	0·71		0·68	
Protein N . .	0·449		0·145	
Non-protein N * .	0·042		0·047	

* Nitrogenous extractives.

The caseinogen-lactalbumin ratios are roughly 3 : 1 and 1 : 2, and the protein-non-protein nitrogen ratios 11 : 1 and 3 : 1 for cow's milk and human milk respectively. Analyses of the isolated proteins for amino-acids revealed significant differences both in the caseinogens and the lactalbumins of the two milks. There is evidence that human caseinogen is more readily digested ; human milk protein is also more digestible because lactalbumins are more easily hydrolysed than caseinogens.

So far we have considered " mature " milk, that is, milk secreted some time after parturition. The secretion of the mammary gland during the first few days of lactation, **colostrum,** differs from " mature " milk not only in appearance—it is yellower and less " milky "—but in composition. Human colostrum contains less fat, more salts and at least twice as much protein. This protein has a high percentage of globulin which causes the milk to coagulate on boiling. (Mature milk contains only very small amounts of globulin.) This globulin is said to be identical with serum euglobulin. About three days after parturition ordinary milk begins to appear and the composition of the secretion gradually changes during the next month (transition period) to that of mature milk.

Milk Concentrates.

Condensed or **evaporated** milk is made by removal of water by evaporation *in vacuo*. Vitamins A and B are preserved. The product is sold as unsweetened condensed milk. Sweetened condensed milk is usually made by adding an amount of cane sugar equal to the total solids of the unsweetened condensed milk.

Dried milk is prepared either by passing a film of milk over hot rollers or spraying milk into hot air so that the water is rapidly evaporated. The first process preserves the vitamins, the second tends to destroy them. The dry powder contains about 5% of water and is practically sterile. Many infant foods have dried milk as a basis. The war-time dried " Household " milk was dried separated milk containing very little fat.

Products Derived from Cow's Milk

Cream. If milk is left to stand, the fat tends to rise to the top. If the two layers are separated, the products are known as **skim cream** and **skim milk.** The former contains about 20% of fat, the latter 1%. A better separation is obtained by using a centrifugal machine. The products are ordinary or separated **cream** and **separated milk.** The fat contents are approximately 45% and 0·3%. In **clotted cream** (Devonshire cream) the separation of the fat is assisted by warming and is more complete ; this type of cream contains nearly 60% of fat. Creams, however, are not only fat and water. They should be regarded as milks in which varying amounts of water have been replaced by fat, for they contain protein, lactose and salts in percentages near to those of milk.

Butter. Butter is formed by churning cream. This causes the fat globules to run together and form a solid mass, leaving a fluid called **butter-milk,** which contains most of the water, protein, lactose and salts of the cream. Butter is not, however, a pure fat. It contains, in addition to about 12% of water, small amounts of caseinogen and lactose. The nature of the fatty acids has been described under milk fat. The characteristic smell of rancid butter is mainly due to butyric and caproic acids. The former is rarely found in other fats. The colour of butter is mainly due to carotene. The total vitamin A + carotene of different samples of butter is fairly constant and sufficient for butter to be classed as a rich source of vitamin A. Butter is poor in vitamin D, especially in winter.

Butter-milk corresponds in composition to milk from which most of the fat has been removed. The fat content is about 0·5%. It thus forms a cheap source of protein of good nutritive value.

Margarine. It is convenient to compare margarine with butter here, although it is not a milk product. Margarine is formed by hydrogenation of unsaturated fats, usually vegetable oils. In general composition it is similar to butter, although the fat does not contain the lower fatty acids of butter ; this probably accounts for the difference in flavour. It keeps better than butter and has a nutritive value only very slightly less than butter. Margarine is devoid of vitamins, but vitamins A and D are usually added so as to give a product with the vitamin content of summer butter. (See p. 440).

PERCENTAGE COMPOSITION OF MILK PRODUCTS [1]

	Protein	Fat	Carbo-hydrate	Calories per 100 g.
Milk (whole)	3·3	3·6	4·8	67
,, (skim)	3·6	0·8	4·6	41
,, (separated)	3·4	0·3	5·1	38
,, (dried)	24·5	24·2	35·1	469
,, (,, separated, "Household")	35·8	0·7	47·9	341
,, (condensed, unsweetened)	8·3	12·4	16·0	215
,, ,, sweetened)	9·7	11·0	53·3	361
Cream (40%)	2·1	41·0	1·5	396
,, Devonshire	4·4	57·4	2·0	560
Butter	0·2	83·0	—	773
Margarine	0·2	84·8	—	789

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[1] The food analyses in this and the following tables are mostly quoted from Mottram and Radloff's compilation (Ref. 75). The values refer to edible portions. For mineral contents see Ref. 75 or 76, and for vitamins the tables at the end of this chapter.

Cheese

Cheese is made by separating the caseinogen of milk, more or less entangled with the fat, and ripening the mass by the action of bacteria. The caseinogen is precipitated either by clotting with rennet or by curdling with an acid such as vinegar. The former method carries down nearly all the fat, the latter not so much. The fat content of cheese will also depend upon whether it is made from ordinary milk, skim milk or milk with cream added. The clotted or curdled mass of caseinogen and fat is squeezed to remove whey. If great pressure is used a " hard " cheese which keeps well is formed, *e.g.*, Cheddar ; if the curd is not appreciably compressed a " soft " cheese is produced. After removal of the whey the curd is allowed to ripen at a suitable temperature under the influence of bacteria which are peculiar to the districts in which the cheeses are made. The characteristic flavours of different cheeses are attributed to products formed from caseinogen by the particular bacteria. While most cheeses are made from cow's milk, some are made from the milk of other animals, *e.g.*, goat and ewe. The cheeses sold in small portions wrapped in tin-foil have been prepared by melting and pasteurising ; they keep well without changing their flavour because they are sterile.

The composition of cheese is roughly equal parts of water, protein and fat with a small percentage of mineral matter which is largely calcium and phosphate. Values which cover most cheeses are water 25%–40%, protein 15%–40%, fat 15%–40%, carbohydrate less than 3%, ash 1·5%–6%. Protein is, perhaps, more accurately called nitrogenous matter, since in some cheeses a considerable amount of the original protein of the milk has been partly changed into proteoses, peptones, amines and ammonia.

PERCENTAGE COMPOSITION OF CHEESES [1]

	Protein	Fat	Carbohydrate	Calories per 100 g.
Cheddar	25·2	32·1	3·4	416
Cheshire	25·6	31·4	1·9	405
Dutch	26·0	15·3	1·8	256
Gorgonzola	24·0	31·9	1·4	400
Stilton	19·4	40·8	2·4	469

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[1] See footnote to table on p. 418.

EGGS

A hen's egg consists of approximately 30% yolk, 59% white and 11% shell. In the edible part there is 15% protein, 10·5% fat and 1% ash.

The **white** is essentially a solution of proteins and salts. The greater part of the protein is **ovalbumin,** a typical albumin. The other proteins are another albumin, **conalbumin,** a globulin, **ovoglobulin,** and a glycoprotein, **ovomucoid.** The pale yellow colour of egg-white is partly due to riboflavin.

The **yolk** is more concentrated, containing only 51% of water. The chief constituents are fat and protein. There is about 1% of mineral matter and small amounts of extractives. The " fat " contains nearly 30% of lecithin and other phospholipides and 4% of cholesterol. The proteins are **vitellin,** a phosphoprotein resembling caseinogen and a globulin, **livetin.** The former predominates (3·6–1). The vitellin and lecithin are associated in a loose (physical ?) combination, *lecithovitellin.* The yolk is well supplied with mineral matter, especially calcium, iron and phosphate. Much of the phosphate is present in the phospholipides and vitellin. The yolk is rich in vitamins A, B_1, riboflavin and D, but not C.

PERCENTAGE COMPOSITION OF EGGS [1]

	Protein	Fat	Carbohydrate	Calories per 100 g.
Whole without shell	12·3	11·3	1·6	162
White	10·7	0·1	1·4	51
Yolk	15·5	33·3	2·1	381
Dried egg	45·8	42·0	3·2	574

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[1] See footnote to table on p. 418.

FLESH FOODS

MEAT

The composition of fresh skeletal muscle is given on p. 183. During hanging nearly all the carbohydrate disappears, and acids, such as lactic acid and acid phosphates (from the organic phosphates), are formed. These acids assist the conversion of collagen to gelatin when the meat is cooked. During cooking, by whatever method, there is shrinkage due to coagulation of protein and loss of water. In boiling, salts, gelatin and extractives are lost. This loss is reduced in processes in which the water is removed by evaporation, *i.e.*, roasting and frying and grilling. Contrary to popular belief the loss on boiling is the same, whether the joint be placed in cold water and gradually heated, or plunged directly into boiling water. There is no experimental basis for the theory that quick coagulation of proteins on the surface of a joint prevents the outward diffusion of salts and extractives.

The flavour of meat is attributed to the extractives, that is, the soluble organic substances which can be extracted from meat by boiling water. These are familiar in the dark brown sticky pastes sold as meat extracts. They are of little or no direct nutritive value, but are of benefit in making meat palatable. The nature of the flavoured extractives is unknown (for the others, see p. 185). Differences in flavour have so far defied chemical analysis.

The composition of meat is very variable, depending largely upon the actual cut, *i.e.*, the relative amounts of protein, fat, collagen (gristle) and bone.

Cooking a joint results in a loss of about one-quarter of the water and varying amounts of salts and extractives, so that the percentages of protein and fat are increased by about 50%. Analyses of cooked meats, including ham, bacon and poultry, range from 22% to 30% for protein and 6% to 23% for fat. Some typical values are given in the table on p. 422.

The non-fleshy parts of animals (the so-called offal) are of considerable nutritive value. **Heart** is very similar to meat but rather denser. **Liver** and **kidneys** contain little connective tissue and a higher proportion of nucleoprotein, *i.e.*, a greater purine content. The former contains about 3% of carbohydrate, a peculiarity shared with **tongue**, which, however, differs from liver and kidney in having a low protein content (18%) and high fat content (24%). **Sweetbreads** (either thymus or pancreas) provide a very digestible food rich in nucleoprotein. **Tripe** (ox stomach and intestine cleaned and boiled) contains a large amount of collagen which is easily converted into gelatin. The nutritive value of **brain** is less than would be expected, owing to its imperfect absorption (about 40% is excreted in fæces).

PERCENTAGE COMPOSITION OF COOKED MEATS[1]

	Protein	Fat	Carbohydrate	Calories per 100 g.
Bacon (fried) . . .	24·6	53·4	—	546
Beef (tinned) . . .	22·3	15·0	—	232
,, salt silverside (boiled) .	28·0	16·5	—	269
,, sirloin (roast) . .	26·8	12·3	—	224
,, rump steak (grilled) .	25·2	21·6	—	305
,, steak (stewed) . .	30·8	8·6	—	206
Brain, sheep's (boiled) . .	11·7	6·7	—	110
Chicken (boiled) . . .	26·2	10·3	—	203
,, (roast) . . .	29·6	7·3	—	189
Duck (roast). . . .	22·8	23·6	—	313
Kidney, sheep's (fried) . .	28·0	9·1	—	200
,, ox (stewed) . .	25·7	5·8	—	160
Liver, ox (fried with flour) .	29·5	15·9	4·0	285
Mutton, leg (boiled) . .	25·8	16·6	—	260
,, ,, (roast) . .	25·0	20·4	—	293
Pork, leg (roast) . . .	24·6	23·2	—	317
Rabbit (stewed) . . .	26·6	7·7	—	181
Sausage, Army, 1918 . .	11·2	17·7	15·2	273
Sweetbread (stewed) . .	22·7	9·1	—	178
Tongue, ox (boiled) . .	19·1	23·9	2·3	310
Tripe (stewed) . . .	18·0	3·0	—	102
Turkey (roast) . . .	30·2	7·7	—	195
Veal, fillet (roast) . . .	30·5	11·5	—	232

[1] See footnote to table on p. 418.

The composition of **sausages** is very variable. They consist of meat, and usually very fat meat, to which bread and seasonings are added. There is usually about 50% water, 10% protein, 25% fat and up to 15% carbohydrate.

FISH

Like meat, fish is practically free from carbohydrate. In general the fat content is much less than that of meat, ranging from a trace to 5%. Exceptions are salmon (13%), herrings (10%) and eels (16%). The average composition of steamed white fish (edible parts) is water 75%, protein 22%, fat 1·4%. The water content is greater than that of meat cooked in the same way. The protein yields rather more gelatin than meat, and fewer

Percentage Composition of Cooked Fish[1]

	Protein	Fat	Carbo-hydrate	Calories per 100 g.
Cod (steamed)	18·0	0·9	—	82
Haddock (steamed)	22·0	0·8	—	98
Halibut (steamed)	22·7	4·0	—	130
Herring (fried)	21·8	18·9	—	265
Kippers (baked)	23·2	11·4	—	201
Mackerel (fried)	20·0	11·3	—	187
Plaice (steamed)	18·1	1·9	—	92
,, (fried)	18·0	14·4	7·0	236
Salmon (steamed)	19·1	13·0	—	199
,, (tinned)	19·7	6·0	—	137
Sardines (tinned in oil)	20·4	22·6	—	294
Sole (steamed)	17·6	1·3	—	84
,, (fried)	20·1	18·4	5·4	276

[1] See footnote to table on p. 418.

extractives. A big difference is made if the fish is fried; fat up to about 12% displaces water, and since it is usual to use breadcrumbs, flour or batter, the fried fish as eaten may contain about 5% of carbohydrate.

Oysters may be mentioned as a form in which we consume glycogen. Out of 12% solids in raw oysters, 3·3% is glycogen and 6% protein.

VEGETABLE FOODS

The chief characteristic of vegetable foods is their high carbohydrate and low protein and fat content. In the form in which they are eaten, they usually contain more water than in the raw state. The protein is usually not so completely utilised by the body as is animal protein. Vegetable foods may be grouped as *seeds* (cereals, pulses, nuts), *roots* (roots, tubers, etc.), *leaves* (green vegetables), and *fruits*.

CEREALS

The general composition of crude cereals (oats, wheat, barley, rye, maize, rice) approximates to 11% water, 11% protein, 70% carbohydrate, and 2% mineral matter, with fat varying from 0·5% to 8%. The exact analysis depends not only on the cereal, but on how it has been milled. In flours made from cereals there is usually not more than 3% of cellulose. Oatmeal is the richest in protein and fat, rice the poorest.

The chief **proteins** of cereals are glutelins and gliadins. The latter are liable to be deficient in certain amino-acids (see p. 391). Small amounts of globulins and albumins are usually present. The **fats** contain sufficient olein to make them liquid at ordinary temperatures. The **carbohydrate** is almost entirely starch in the form of grains covered by a thin membrane of insoluble carbohydrate, mainly cellulose. Starch grains are insoluble and indigestible. Cooking, by bursting the cellulose covering of the grains, renders starch soluble and digestible. Small amounts of sugar are often present in cereals. The most abundant **mineral constituents** are calcium and phosphate. The latter is partly in the form of phytic acid (inositol hexaphosphate), which is not utilisable by man and also hinders the absorption of calcium. This substance is responsible for the so-called " anti-calcifying " effect of cereals (see p. 300). Over 60% of the phosphate of oatmeal is in this form. This phosphate must not be counted as available phosphorus.

It has already been mentioned (p. 360) that milling wheat to produce a flour, which keeps well without the fat going rancid, involves the removal from the endosperm of the germ, or embryo, which contains the B and E vitamins, protein, fats and salts. In addition to the germ, which forms about 1½% of the grain, the outer envelope (pericarp), called **bran**, is removed. This contains much of the cellulose of the grain and is especially rich in salts (6%). It has approximately 16% of nitrogenous matter and over 40% of carbohydrate, part of which is of nutritive value. It forms about 13½% of the whole grain. The nutritive value of roller-milled white flour is considerably less than that of wholemeal flour. There is a serious loss of vitamins B and E, and of mineral elements, especially Ca, P and Fe. The quality and the quantity of the protein is slightly diminished. Further losses, of carotene and riboflavin, were caused by chemical bleaching. In the milling of white flour about 30% of the grain is discarded (" offal ") and the flour is described as being of 70% extraction. The loss of vitamins and minerals from wheat can be largely prevented by raising the extraction to 85% (see Fig. 32, p. 408) which retains the germ. This was done compulsorily during the war of 1939–1945, the product being known as *National Flour.*

In the preparation of brown flours, either the germ, or bran, or both, are added to the white flour from the endosperm. The germ is usually sterilised to prevent the fat going rancid and to destroy enzymes liable to cause fermentation ; the bran is boiled with water under pressure to break down the cellulose and liberate the constituents of nutritive value.

Bread. Flour must be cooked to make it easy to digest. The most convenient way is to convert it into bread, which is essentially cooked flour puffed up into a sponge so as to give easy access for digestive juices. The gliadin and glutelin of wheat flour form a gluten which on kneading with water gives a plastic *dough.* In breadmaking this dough is blown up by gas (" rises "), the gluten having sufficient cohesion to retain the gas. By baking the risen dough the entrapped gas is further expanded and the gluten is hardened to give the stable spongy structure of the bread. The gas (CO_2) is produced by the alcoholic fermentation of sugar by yeast mixed with the dough. The form in which the yeast and sugar are added varies in different localities, and this is largely responsible for the different flavours of bread. The sugar may be added as such, or may be formed by the action of an amylase (*e.g.*, in the flour) on the starch of cooked potato pulp. Most of the alcohol formed by the fermentation of the sugar is lost in baking, since new bread only contains about 0·2%.

The baking is done in ovens at about 235° C. This bursts the starch grains and converts some of the starch into soluble starch and dextrins. The exposed surface of the loaf is raised to a temperature approaching that of the oven, and here most dextrin is formed. This is partly converted into a brown caramel which, with decomposition products of protein, gives the crust its characteristic flavour and appearance.

Bread can only be made from cereals having a gluten * which gives a dough with good cohesion. Wheat has the best gluten for this purpose. Oatmeal and rice flour contain practically no gluten and, alone, cannot make bread.

Bread differs from flour in having a higher water content and the starch in a soluble form, a small part of which is dextrin and sugar. There is approximately 40% water, 51% starch (including dextrin and sugar) and 7% protein. About two-thirds of an ordinary loaf is gas.

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* In the formation of a gluten suitable for breadmaking, several factors are concerned, such as the presence of gliadin, glutelin and phosphates, and the pH.

Biscuits are made by baking moistened flour, either alone or mixed with small amounts of milk, butter, sugar, etc. Sometimes gas is produced in the mixture by adding baking powder.* In **cakes** there is a larger proportion of butter and sugar, with the addition of baking powder and eggs.

Oatmeal is richer in protein, fat and minerals than other cereals. The hull of oats is difficult to remove by milling. This accounts for the " roughness " of oatmeal and its higher cellulose content. Oatmeal from which the hull has been entirely removed is termed **groats.** The long boiling needed to soften the cellulose of oatmeal has led to the marketing of various prepared " oats " in which the grains are usually flattened between rollers and partly cooked. The proteins contain all the essential amino-acids and are well absorbed (over 90%), but a large proportion of the phosphorus is in the form of phytin. This disadvantage can be overcome by taking porridge with plenty of milk ; this makes an excellent food.

Of the other cereals, **rye** is frequently used for breadmaking in some countries, although it makes a poor gluten, and therefore a heavy bread. **Barley** and **maize** (Indian corn) do not contain enough gluten to make good bread ; they are sometimes used to adulterate wheat flour. **Rice** is the poorest of all cereals in respect of protein, fat and minerals, and richest in starch.

Cereal Products

Macaroni and **vermicelli** are made by drawing the paste formed by mixing gluten-rich wheat flours with water into tubes which are hardened by drying or slight baking.

Semolina is a granular product of gluten-rich wheat flours.

Pearl barley is the polished grain of barley. When ground to a flour it is described as **patent barley**.

Crispbread is usually prepared from whole rye.

Cornflour is a starch preparation formed by washing away the protein, fat and salts from maize. Only traces of protein and salts are left adhering to the starch grains.

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* Sodium bicarbonate and tartaric acid or acid tartrate, a mixture which evolves CO_2 on wetting. Flour already mixed with baking powder is known as self-raising flour.

PERCENTAGE COMPOSITION OF CEREAL PRODUCTS[1]

	Protein	Fat	Carbohydrate	Calories per 100 g.
Bread (National)	8·5	1·2	51·4	250
,, (white)	8·5	1·1	54·6	267
,, (brown)	6·3	1·1	50·2	239
Biscuits.				
Cream Cracker	8·1	18·4	70·6	494
Digestive	5·6	25·0	64·5	520
Water	11·9	7·3	75·0	425
Osborne	7·4	10·8	79·5	457
Shortcake	8·0	26·4	62·4	534
Flour (National, 85%)	11·7	1·6	71·0	345
Flour (English, 70%)	8·1	1·0	76·2	346
Macaroni	12·8	0·2	75·5	364
Oatmeal	11·9	8·6	70·0	416
Pearl barley	8·0	1·4	77·8	365
Rice	5·9	0·4	80·3	357
Cornflour	0·8	0·1	87·6	363
Corn flakes	10·7	5·2	78·7	415
Crispbread	11·6	1·3	74·8	366

[1] See footnote to table on p. 418.

The various ready cooked breakfast cereals are usually malted wheat or maize suitably shaped and baked.

Malt is made by allowing moistened barley to germinate under controlled temperature conditions. The enzymes formed on germination convert a considerable amount of the starch into dextrin and maltose, and proteins to soluble degradation products. The process is checked by drying when about a quarter of the whole grain has been rendered soluble. Malt is frequently added to cereal preparations to improve their flavour.

PULSES

The chief difference between dried pulses (peas, beans, lentils, etc.) and cereals is the high proportion of protein in the former. The chief protein is a globulin called **legumin.** The protein content of dried pulses is from 20% to 25%, *i.e.*, double that of cereals, which have approximately the same water content (11%). The fat content is usually under 2%. The protein is only well absorbed if taken in finely divided form ; as ordinarily consumed, about 20% is unabsorbed. The *soy* or *soya* bean is peculiar not only in having a much higher protein content, but also a high fat and, therefore, low carbohydrate content. It is much used in the Far East to supplement the deficiencies of rice, and soya bean flour has recently been made available in this country. The oil is used for frying "fish and chips."

Percentage Composition of Pulses[1]

	Protein	Fat	Carbo-hydrate	Calories per 100 g.
Beans, broad (boiled)	4·1	Less than 2%	7·1	46
,, butter (boiled)	19·2		49·8	283
,, French (boiled)	0·8		1·1	8
,, haricot (boiled)	6·6		16·6	95
,, scarlet runner (boiled)	0·8		0·9	7
Lentils (boiled)	6·8		18·3	103
Peas, fresh (boiled)	5·0		7·7	52
,, dried (boiled)	6·9		19·1	107
Soya bean flour (full fat)	40·4	23·5	13·3	426

[1] See footnote to table on p. 418.

NUTS

Nuts are characterised by a high fat and low carbohydrate content. The protein content is slightly lower than that of dried pulses. Raw nuts are not easily digested. This difficulty is largely overcome by grinding and cooking the nuts as in vegetarian cookery.

Percentage Composition of Nuts[1]

	Protein	Fat	Carbo-hydrate	Calories per 100 g.
Almond	20·5	53·5	4·3	599
Brazil nut	13·8	61·5	4·1	645
Chestnut	2·3	2·7	36·6	185
Cob nut	9·0	36·0	6·8	400
Coconut	3·8	36·0	3·7	366
Peanut *	28·1	49·0	8·6	606
Walnut	12·5	51·5	5·0	479

* The peanut is strictly a pulse, although it has a composition typical of nuts.

[1] See footnote to table on p. 418.

ROOTS AND TUBERS

The most important tuber used in this country is the **potato**. In its composition the richness in starch is most noticeable. It provides, in fact, the chief commercial source of starch. Only about half the total nitrogen is present as protein (chiefly the globulin *tuberin*). The remaining nitrogen is in the form of simple soluble nitrogenous compounds such as asparagine. Potatoes are dietetically valuable as foods rich in carbohydrate which can with advantage substitute sugar and cereals. They are free from the suspicion of causing predisposition to dental caries laid upon sugar and cereals, and form a valuable source of iron and vitamin C, which is largely retained after cooking.

The polysaccharide of **Jerusalem artichokes** is the fructosan inulin, which is probably not utilisable by man to any significant extent. There is, in addition, about 7% carbohydrate (sugar) available to the body. The nitrogen distribution is similar to that of the potato.

With the exception of parsnips, the common roots (**turnips, swedes, beetroots, carrots**) are almost free from starch. Their

PERCENTAGE COMPOSITION OF ROOTS, TUBERS, ETC.[1]

	Protein	Fat	Carbohydrate	Calories per 100 g.
Potato (boiled)	1·4	—	19·7	87
,, (roast)	2·8	1·0	27·3	133
,, (chips)	3·8	9·0	37·3	252
Artichoke, Jerusalem (boiled)	1·6	—	3·2	20
Beetroot (boiled)	1·8	—	9·9	48
Carrots (boiled)	0·6	—	4·3	20
Onions (boiled)	0·6	—	2·7	14
,, (fried)	1·8	33·3	10·0	358
Parsnips (boiled)	1·7	—	13·5	62
Radishes (raw)	1·0	—	2·8	16
Swedes (boiled)	0·9	—	3·8	19
Turnips (boiled)	0·7	—	2·3	12
Arrowroot	0·2	0·03	88·4	364
Sago	0·2	0·02	87·7	361
Tapioca	0·2	0·05	87·7	361

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[1] See footnote to table on p. 418.

calorie value is almost entirely due to sugars (cane sugar, fructose and glucose). Carrots, which are richest in sugars (10%), are a valuable source of carotene, the equivalent of vitamin A. Roots may contribute valuable salts, but the protein and fat are of negligible value.

Arrowroot is starch prepared from the rhizomes of West Indian plants of the *Maranta* species. It contains traces of protein and salts.

Tapioca is a similar product from the roots of *cassava* plants (S. America).

Sago is a starch prepared from the pith of the *sago palm.*

GREEN VEGETABLES

The chief nutritive value of green vegetables is their content of vitamins (A, B's and C) and salts. Their calorific value is small and mostly due to sugar. The cellulose provides "roughage" to stimulate peristalsis. The water content is higher than that of any of the groups described so far; cooking increases it. Much of the nitrogenous matter is non-protein. Green vegetables are essentially protective foods, and, for this reason, important articles of diet. Cooking causes loss of B and C vitamins (see p. 440); uncooked salads are, therefore, more valuable as protective foods.

PERCENTAGE COMPOSITION OF GREEN VEGETABLES [1]

	Protein	Fat	Carbohydrate	Calories per 100 g.
Asparagus (boiled)	1·1	Very small amounts	3·4	18
Brussels sprouts (boiled)	2·4		1·7	17
Cabbage (boiled)	0·8		1·3	9
Cauliflower (boiled)	1·5		1·2	11
Celery (raw)	0·9		1·3	9
,, (boiled)	0·6		0·7	5
Greens (boiled)	1·7		0·9	11
Leeks (boiled)	1·8		4·6	26
Lettuce (raw)	1·1		1·8	12
Marrow (boiled)	0·4		1·4	7
Spinach (boiled)	5·1		1·4	27
Watercress (raw)	2·9		0·7	14

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[1] See footnote to table on p. 418.

FRUITS

Fresh fruits are also essentially protective foods, although the energy value is about twice that of green vegetables. This is due to sugars and starch, the protein and fat usually amounting to less than 0·5%. Many fruits contain pentoses and pectins. The latter cause the "setting" of jams and jellies made by boiling fruits with sugar. The various organic acids present (tartaric, malic, citric), lower the pH sufficiently to enable the pectin to set. (Pectin does not form a jelly except in the presence of a high concentration of sugar and at a pH in the region of 3·5.) Neither pentoses nor pectins are utilised by the body.

Cooking involves a loss of most constituents unless the juice is also consumed ; then the most serious loss is vitamin C. Cooking makes fruits more digestible by softening the cellulose.

Some fruits, however, have a definite energy value, notably the **banana,** which is peculiar in containing starch as well as sugar. It also has a somewhat higher protein content. The starch is

PERCENTAGE COMPOSITION OF FRESH FRUITS[1]

	Protein	Fat	Carbohydrate	Calories per 100 g.
Apples	0·3	Very small amounts	11·7	51
Apricots	0·6		6·7	31
Bananas	1·1		19·2	83
Blackberries	1·3		6·4	32
Cherries	0·6		11·9	50
Grapes	0·6		15·5	66
Grapefruit	0·6		5·3	24
Lemon juice	0·3		1·6	8
Melon (Cantaloupe)	1·0		5·2	26
Oranges	0·8		8·5	38
Orange juice	0·6		9·4	41
Pears	0·2		10·4	44
Plums (dessert)	0·6		9·6	41
,, (cooking)	0·6		6·2	28
Raspberries	0·9		5·6	26
Rhubarb (stewed)	0·6		0·7	5
Strawberries	0·6		6·2	28
Tomatoes	0·9		2·8	15

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[1] See footnote to table on p. 418

said to be very easily digested. Flours made from bananas contain 80% carbohydrate and 4% protein and have been used for invalid diets, especially for young children.

Dried fruits may be valuable foodstuffs, although vitamin C is usually lost on drying. **Dates,** in fact, form the staple diet of desert tribes. The carbohydrate of dates (75%) is all sugar, not starch. The protein content is rather low (2%). Other dried fruits of similar composition are figs, prunes and raisins.

PERCENTAGE COMPOSITION OF DRIED FRUITS [1]

	Protein	Fat	Carbohydrate	Calories per 100 g.
Apricots (dried)	4·8	Very small amounts	43·4	198
Currants	1·7		63·1	266
Dates	2·0		63·9	270
Figs (dried)	3·6		52·9	245
Prunes	2·4		40·3	175
Raisins	1·1		64·4	269
Sultanas	1·7		64·7	272

[1] See footnote to table on p. 418.

SUGAR

The dietetic value of refined cane sugar is discussed on p. 396. Other forms of sugar frequently consumed are :—

Beet-sugar, identical with sucrose (cane sugar), if fully refined.

Maple-sugar, crude sucrose from the sap of the sugar maple.

Treacle, Golden Syrup, Molasses, crude partly hydrolysed

PERCENTAGE OF CARBOHYDRATE IN SUGARS, ETC.[1]

	Carbohydrate	Calories per 100 g.
White sugar	100·0	410
Demerara sugar	98·0	402
Golden syrup	81·0	332
Treacle	59·9	252
Honey	71·4	294
Jam	69·4	286

[1] See footnote to table on p. 418.

sucrose, formed as by-products in the manufacture of crystalline sucrose.

Barley Sugar. Sucrose heated until it melts to a yellow liquid and cooled. It does not crystallise after this treatment.

Caramel. Sucrose heated until the colour darkens.

Honey. A crude mixture of glucose and fructose.

Values for the relative sweetness of different sugars determined by Biester, Wood and Wahlin on the basis of the minimum concentration of sugar in water reported as sweet by at least twenty tasters are :—

Fructose	173	Galactose	32
Invert Sugar	130	Maltose	32
Sucrose	100	Lactose	16
Glucose	74		

TEA, COFFEE, COCOA

Tea. The black tea ordinarily sold consists of the leaves of young shoots of the tea plant which have been fermented and dried by heat. In green tea the fermentation is omitted. The infusion consumed is of negligible calorific value. Its chief interest lies in two constituents, a stimulant and diuretic, **caffeine,*** and an astringent, **tannic acid.**† The former, which is present to the extent of 2%–4% in the dry tea, is readily soluble and is quickly extracted when tea is made. The latter (5%–15%) is less soluble and only passes into the infusion slowly ; this accounts, in part, for the increased bitterness of the liquid which has stood over the leaves for some time. A cup of strong tea contains about 0·1 g. (1½ grains) of caffeine. Strong tea, largely owing to the tannic acid, retards gastric digestion.

Coffee. Coffee is the roasted seed of the cherry-like fruit of

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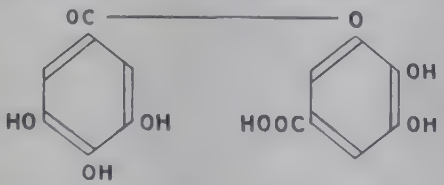
* 1, 3, 7-Trimethyl xanthine (p. 114).

† Tannic acids are compounds resembling digallic acid



Digallic Acid

often present in plants as glucosides, called *tannins.* (This name is, however, sometimes used for the acids.) The exact nature of tea tannic acid is unknown. Tannic acids form insoluble compounds with proteins.

Caffœa arabica. The aroma is due to an oil, **caffeol,** formed when the beans are roasted. Like tea, the infusion, although containing more solids, is of little calorific value and contains caffeine and tannic acid in amounts of the same order as in tea infusion. Neither tea nor coffee, unless they are taken with milk and sugar, can be regarded as foods. Their value is largely due to the pharmacological properties of caffeine. Coffee is frequently mixed with the caffeine-free roasted root of the wild endive, *chicory*.

Cocoa. The seeds obtained from the pods of the cacao tree (*Theobroma cacao*) after fermentation and roasting are known as cocoa-nibs. The nibs contain about 50% of fat, part of which is removed by pressure (**cacao butter**). The finely ground residue is known as cocoa * and contains from 25% to 30% of fat. Sugar and starch are added in some marketed brands. There is some caffeine (up to 0·5%) in cocoa, but more (1–3%) 3,7-dimethyl xanthine, **theobromine,** which has similar pharmacological properties. Although its analysis suggests a high nutritive value, cocoa as a beverage is of little importance, since so little is consumed. The milk and sugar taken with it provide most of the nourishment.

Chocolate consists of ground cocoa-nibs mixed with sugar. Starch and flavouring are frequently added. The powder, owing to its high fat content, melts easily and can be cast into bars, etc. The bloom of cheap chocolates is due to sugar separating from the mixture. The basis of the so-called "cream" of chocolate creams is cane-sugar and glucose.

PERCENTAGE COMPOSITION OF COCOA AND CHOCOLATE [1]

	Protein	Fat	Carbohydrate	Calories per 100 g.
Cocoa	18·1	26·8	40·3	489
Chocolate	4·8	31·1	59·9	555

[1] See footnote to table on p. 418.

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* Cocoa is an English corruption of the word cacao. It must be distinguished from the cocoa palm yielding coconuts and from coca, the source of cocaine.

ALCOHOL

Alcohol has an energy value of 7 Calories per gram, *i.e.*, greater than carbohydrate. Since alcohol can yield this energy in the body it must be admitted as a food,* although its use is somewhat restricted owing to its pharmacological action. It is chiefly consumed in the form of beers, wines, spirits or liqueurs.

Beers are formed by fermentation of malt and contain from 4% to 8% of alcohol by volume. Fermentation is not complete and there is usually some sugar (up to 2%) and dextrin (up to 4%) left, in addition to small amounts of protein.

Wines are the product of fermentation of fruit juices (usually grape). The alcohol content is from 10% to 20% by volume, and sugar from 0·1% to 4% (Port 7%). The chief chemical characteristic is the presence of a large number of organic acids (*e.g.*, malic, tartaric, succinic, etc.) as well as several alcohols and esters. Cider and perry can be considered as wines of low alcohol content (3–8%) and higher sugar content.

Spirits are formed by distillation of various fermented products. Whisky and gin can be regarded as distilled beer, brandy as distilled wine. They are, therefore, practically free from sugar or solid matter. The alcoholic content is from 30% to 50% by volume.

Liqueurs are essentially alcohol sweetened with cane sugar and flavoured with aromatic herbs or essences. The sugar content is usually in the region of 30% and alcohol 35%–55% by volume. Absinthe and brandy are sugar free.

AVERAGE PERCENTAGE COMPOSITION OF ALCOHOLIC LIQUORS

	Alcohol (by volume)	Alcohol (by weight)	Carbohydrate	Calories per 100 c.c.
Beers . . .	5	4	6	52
Wines . . .	12	10	2	78
Spirits . . .	40	33	0	91
Liqueurs . . .	45	38	30	386

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* As Dodds has remarked, " A double whisky has the same *energy* value as three eggs."

VITAMIN CONTENT OF FOODS (78, 79, 85)

Relatively accurate evaluation of the vitamin potency of foods only became possible when vitamins had been sufficiently purified to provide stable preparations which were suitable for distribution and use as standards of comparison throughout the world, for it is essential in assaying vitamin potency on animals that a simultaneous comparison be made against an approved standard. Comparison with a standard is equally desirable in chemical methods of assay. Results obtained in different countries can only be accurately compared if they have been related to some common standard. Official International standards and units were adopted for vitamins A, B_1, C and D under the auspices of the League of Nations in 1931, revised in 1934 and 1938, and are used at the present time. The International Units are :—

Vitamin A. The activity of 0·0006 mg. of the International Standard Preparation of pure β-carotene.

Vitamin B_1. The activity of 3 micrograms of the International Standard Preparation of pure vitamin B_1.

Vitamin C. The activity of 0·05 mg. of pure *l*-ascorbic acid.

Vitamin D. The activity of 1 mg. of the International Standard solution of irradiated ergosterol (0·000025 mg. of pure calciferol).

No unit has been established for riboflavin (lactoflavin).

The vitamin content of some of the foods described in this chapter are given in the following tables. Only values which have been obtained since 1931 by direct comparison with the International standard or a reliable sub-standard, or by chemical methods of acknowledged reliability have been included. Except where only one is available, the values are given as ranges. In most instances these ranges indicate such great natural variation in vitamin activity that the expression of the " average vitamin potency " of a food would obviously be very misleading. The carotene values in the tables are expressed in International units for the reader's convenience, but are given with the warning that there is some doubt as to whether carotene is always quantitatively converted to vitamin A in the animal. (The vitamin A values obtained by biological assay should not be compared with the carotene values, since in most instances the assays were made on different samples of the foodstuff.) The foods in the tables have

In International Units per 100 g. (or 100 ml.) unless stated

Material	Total Vitamin A Potency by Biological Assay	Vitamin A Content (Spectro-photometric)	Carotene Content	Vitamin B_1 Content (Biological Assay)	Riboflavin (Lactoflavin) Content in mg. per 100 g.	Ascorbic Acid Content	Vitamin D Content (Biological Assay)
Human milk	200–500	160–270	—	—	—	24–216	—
Cow's milk							
Raw	140–700	133–220	—	23	0·1–0·3	6–58	2·4–3·8 (summer) 0·3–1·7 (winter)
Pasteurised	70–550	—	—	—	—	17–66% loss	1·7 (winter)
Boiled	—	—	—	—	—	33–75% loss	—
Dried	428	—	—	—	—	—	—
Cream	—	—	—	—	—	—	50
Butter-fat	4,000–8,500	740–3,865	240–3,750	—	—	—	8·5–400
Butter	850–7,500	—	100–700	—	0·008	—	0–130
Cheese							
Cheddar	5,500	—	—	0	—	—	—
Gorgonzola	—	—	—	30	—	—	—
Gruyère	400	—	—	—	—	—	—
Egg, white	—	—	—	—	0 4–0·5	0	—
,, yolk	1,600–8,800	2,300–3,840	230–6,660	100	0·5–0·6	0	150–500
Meat							
Beef, lean	—	60	—	30–100	0·04–0·35	32–44	—
Mutton, lean	—	31	—	60–119	0·07	50	—
Heart, ox	—	—	—	—	0·35	92	—
Kidney, sheep	—	—	—	190	—	—	—
Liver, ox	12,000–41,800	—	—	150 (cooked)	0·1–3·0	480–1,360	40–50
Pancreas, ox	—	—	—	—	—	244	—
Rabbit, muscle	—	—	—	—	0·05–1·2	8·4–30	—
Fish							
Cod	—	—	—	40	0·05–0·3	—	—
Oyster	420	—	—	—	0·46	60	5
Salmon, tinned	—	—	—	—	—	—	600–800
Sardine, tinned	—	—	—	30	—	—	—
Cod-liver oil	40,000–400,000	—	—	—	—	—	2,000–30,000
Halibut-liver oil	1,920,000–36,000,000	—	—	—	—	—	20,000–400,000
Tunny-liver oil	512,000–8,000,000	—	—	—	—	—	85,000–25,000,000

Where a blank is left in the table it does not mean that the vitamin is absent, but that its presence has not been quantitatively investigated. Values are quoted from Boas-Fixsen and Roscoe, Ref. 78.

VITAMIN CONTENT OF FOODS OF PLANT ORIGIN

In International Units per 100 *g.* (*or* 100 *ml.*) *unless stated*

Material	Vitamin A Potency by Biological Assay	Carotene Content	Vitamin B_1 Content (Biological Assay)	Riboflavin (Lactoflavin) Content in mg. per 100 g.	Ascorbi Acid Content
Cereals					
Bread (white)	—	—	12–30	0·07	—
,, (wholemeal)	—	—	75–130	—	—
,, (brown)	—	—	50–80	—	—
,, (germ)	—	—	80–170	—	—
Wheat (whole grain)	—	170–760	118–340	0·02	600
,, (germ)	650	—	400–2,200	0·033	—
,, (white flour)	—	—	0–30	—	—
Semolina	—	485	—	—	—
Maize	—	17–1,500	5–60	0·1	23
Oatmeal	—	—	325	—	—
Rice (brown)	—	57	20–90	0·07	—
,, (polished)	—	0	0	0·08	—
,, (polishings)	—	—	560–760	—	—
Pulses					
Beans:					
Broad (seeds only)	—	—	—	—	448–680
Butter (dry)	—	—	160	—	—
French	—	367–668	—	0·03–0·57	36–300
Haricot	—	—	52–120	—	—
Scarlet runner	600	—	25–75	—	20–444
Lentils (dry)	—	88–750	40–210	0·068	60
,, (sprouted)	—	—	—	—	300
Peas (fresh)	700	232	98–280	0·01–0·28	96–800
,, (dried)	—	—	40–480	0·08	0–54
,, (sprouted)	—	—	—	0·28	160–1,72
Nuts					
Almond	—	—	80	—	0–386
Chestnut	—	—	90	—	646–1,00
Coconut	—	0	10–20	0·1	8–268
Hazel	—	—	200	—	300
Peanut	5–47	105	100–320	0·17–0·46	200
Walnut	—	1,600	—	—	600
Red palm oil	60,000–190,000	40,000–510,000	—	—	—
Roots, Tubers, etc.					
Potato	25	47–93	30–60	0·0075–0·01	220–720
Artichoke, Jerusalem	—	—	—	—	116
Beetroot	50 (cooked)	0	70 (boiled)	0·055	54–200
Carrot	1,900	3,300–16,000	60	0·02	20–620
Onion	—	42	40	—	52–300
Parsnip	200	50	—	—	100–800
Radish	—	—	6–60	0·02	248–400
Turnip	—	0	40	—	340–868
Sago	—	0	0	—	—

Where a blank is left in the table it does not mean that the vitamin is absent, but that its presence has not been quantitatively investigated. Values are quoted from Boas-Fixsen and Roscoe, Ref. 78.

VITAMIN CONTENT OF FOODS OF PLANT ORIGIN—*continued*

Material	Vitamin A Potency by Biological Assay	Carotene Content	Vitamin B_1 Content (Biological Assay)	Riboflavin (Lactoflavin) Content in mg. per 100 g.	Ascorbic Acid Content
Green Vegetables					
Asparagus	—	—	—	—	240–1,430
,, tips	—	—	—	—	420–3,310
Brussels sprouts	—	50–1000	60	—	143–2,920
Cabbage	900	—	25–80	0·05	400–2,480
Cauliflower	—	Head 63	—	0·08	—
		Leaves 5,500–6,500	110	—	380–2,020
Celery (stalk)	50	Leaves 9,600–12,500	Trace	—	20–114
Leek	—	—	—	—	80–660
Lettuce	—	2,500–4,000	90	0·1	10–440
Marrow	30	0	10–20	—	54–356
Spinach	13,000	4,400–10,800	20–70	0·057	120–2,480
Watercress	1,000	—	60	—	480–1,520
Fruits					
Apple	40	—	40	—	2–400
Apricot	—	3,000–3,800	—	—	16–320
Banana	80–335	400	17–60	0·0075–0·018	20–300
Cherry	—	—	—	—	62–340
Currant, black	300–500	—	0–20	—	1800–6880
Grape	—	25	—	—	20–80
Grapefruit, juice	—	—	—	—	520–1,300
,, pulp	—	—	40	—	—
Lemon, juice	—	—	—	0·003	516–1,420
Melon (Cantaloupe)	—	—	10–20	0·065	300–1,060
Orange, juice	300	500–670	—	0·0069–0·0089	440–1,780
,, pulp	—	—	40	—	320–940
Pear	—	134	30	0·007–0·03	20–200
Plum	—	0·380	40	0·025	10–300
Raspberry	—	—	25–35	—	610
Rhubarb (stalk)	—	—	—	—	118–734
Strawberry	—	—	—	—	920–1,550
Tomato	3,000	Flesh 23,500–60,000 Juice 530–980 Skin 440,000–1,480,000	18–40	0·05–0·071	258–780
Dried Fruits					
Apricot	—	8,500–9,200	—	0·057	—
Date	—	1,000	—	—	—
Fig	—	—	15–100	—	—
Prune	—	—	66–90	—	20
Raisin		—	50–100	—	—
Sultana	—	—	60	—	—
Yeast, dried, baker's	—	—	310–1,000	2·5–3·6	—
,, dried, brewer's	—	184	600–12,000	1·8–3·0	—

Where a blank is left in the table it does not mean that the vitamin is absent, but that its presence has not been quantitatively investigated. Values are quoted from Boas-Fixsen and Roscoe, Ref. 78.

been arranged in the order in which these foods have been considered previously in this chapter. It will be recalled that vitamin A itself, as distinct from the pro-vitamin A, carotene, is not found in plant material. Vitamin D is also practically absent from non-irradiated foods of plant origin, so that in the second table two columns have been eliminated.

Average vitaminised margarine contains about 1,600 units of vitamin A and 110 units of vitamin D per 100 g.

Effect of Cooking on Vitamin Content of Foods (30, 85)

The vitamin content of cooked and canned foods is given in Ref. 85. The effect of cooking on green vegetables may be summarised as follows :—

Vitamin A is unlikely to suffer damage.

Water-soluble B and C vitamins are likely to be lost by diffusion into the soaking or cooking water.

Raw vegetables contain enzymes which destroy vitamins. These act if the vegetables are left to stand after bruising or cutting up. They act more rapidly if the temperature is raised, and are only themselves destroyed at about 80° C. It is, therefore, better to cook vegetables by plunging into boiling water or hot fat rather than by raising to the boil from cold. In the latter method there may be considerable destruction of vitamin before the enzyme is destroyed.

Water soluble vitamins are destroyed by prolonged heating and the vitamin content of cooked foods diminishes if they are left standing. These losses are reduced by adding salt or sugar before cooking.

Vitamins B_1 and C are more stable in acid solution. Alkali, *e.g.*, sodium bicarbonate, hastens the destruction of vitamin B_1.

Vitamin values for a number of war-time foods are to be found in Ref. 79.

CHAPTER XXXII

EXCRETIONS

1. URINE (1, 3, 5, 8)

THEORIES of renal secretion and comparisons of the concentrations of the various constituents of urine and plasma are fully described in textbooks of Physiology. Here we shall confine our attention to the physical properties and chemical composition of urine.

Volume

The volume of urine secreted during twenty-four hours by a healthy adult may vary within wide limits. The amount of water excreted will obviously depend upon the amount ingested and the amount excreted in perspiration. These factors are a reflection of the habits and occupation of the individual and of the climate in which he lives. In this country an adult ordinarily excretes from 1,000 c.c. to 1,800 c.c. of urine in the twenty-four hours. The average used for rough calculations is 1,500 c.c. (or 50 oz.) containing 60 g. (or 2 oz.) of solids. The amount formed during the night is less than 500 c.c.

We know from personal observation that we pass more urine after taking a large amount of water in the form of drinks, soups or wet foods than if we take our food relatively dry. A high protein diet causes increased excretion because the urea formed in its catabolism has a diuretic action. The diuretic action of tea, coffee and cocoa is mainly due to caffeine or theobromine. The decreased volume of urine in hot weather is due to an increased loss of water by perspiration. Nervousness or excitement causes increased urinary volume as well as increased frequency of micturition.

The normal output of urine is affected by certain pathological conditions. *Increases* are observed in diabetes insipidus, diabetes mellitus, and certain types of kidney disease (*e.g.*, amyloid disease, chronic interstitial nephritis) and *decreases* in acute nephritis, fevers, diseases of the heart, and conditions involving diarrhœa and vomiting.

Specific Gravity

The specific gravity of a twenty-four-hour specimen of urine usually lies between 1,010 and 1,025. In health, samples passed at different times of the day will vary with the food and water intake and the activity of the individual; at night, when these variable factors are largely eliminated, a urine of roughly constant composition and fairly high specific gravity (*e.g.*, 1,020) is formed. In conditions such as chronic interstitial nephritis, the concentrating power of the kidneys may be so reduced that a urine of roughly constant composition and low specific gravity (*e.g.*, 1,010) may be formed throughout the twenty-four hours. In health, extreme values for a selected specimen might be 1,003 after a very large water intake and 1,040 after very profuse sweating. In disease, the excretion of abnormal substances such as albumin or glucose may raise the specific gravity (*e.g.*, diabetes mellitus, 1,025 to 1,040).

Colour

The depth of the amber colour of normal urine is roughly proportional to the specific gravity. Very dilute urines may be almost colourless. The chief pigment of urine, **urochrome,** is a nitrogenous compound of unknown constitution * and uncertain origin. The daily excretion of urochrome is very constant, and, according to Drabkin, proportional to the basal metabolism of the individual. Traces of other pigments have been detected, **coproporphyrin** (p. 174), **urobilinogen** (p. 176) and **uroerythrin** (nature unknown). The oxidation of the colourless urobilinogen to urobilin occurs on standing, and may be the cause of the deepening of the colour of concentrated urines on standing.

Abnormally, urines of almost any colour may be observed. Ingestion of food (*e.g.*, sweets) coloured with aniline dyes frequently results in the elimination of the dye in the urine, either unchanged, or in some altered form. More rarely, ingestion of naturally coloured foods (*e.g.*, beetroot, or blackberries) may cause a reddish urine. Pathologically, abnormal constituents may cause pigmentation of the urine. Some examples are given in the table. The urine in these conditions is not always pigmented.

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* The available evidence suggests that urochrome is a compound of a polypeptide with urobilinogen or urobilin. It contains sulphur.

PIGMENTATION OF URINE IN PATHOLOGICAL CONDITIONS

Colour	Cause	Condition
Nearly colourless.	Dilution of normal pigments.	Diabetes insipidus, chronic interstitial nephritis, granular nephritis, diabetes mellitus (untreated).
Orange.	Concentration of normal pigments.	Fevers.
Orange-brown (with greenish tinge).	(*a*) Urobilin.	Excessive hæmolysis, *e.g.*, acholuric jaundice, pernicious anæmia.
	(*b*) Bilirubin.	Obstructive jaundice, certain liver diseases.
Red-brown (often "smoky" with R.B.C.).	(*a*) Blood corpuscles.	Hæmorrhage in urinary tract.
	(*b*) Hæmoglobin.	Paroxysmal hæmoglobinuria, blackwater fever.
	(*c*) Uroporphyrin.	Congenital porphyrinuria.
Brown.	Methæmoglobin.	Blackwater fever, severe toxic conditions.
Brownish-black after exposure to air.	(*a*) Melanin.	Melanotic sarcoma of liver.
	(*b*) Homogentisic acid.	Alkaptonuria.
	(*c*) Hydroquinone, etc.	Phenol poisoning.
Dirty green or blue after exposure to air.	Excess indican.	Cholera, typhus, gangrene of lung.

The colour of urine may also be affected by sufficiently large doses of drugs, amongst them the following :—

Colour	*Drug*
Nearly colourless.	Diuretics.
Reddish-orange (in alkaline urine).	Rhubarb, senna.
Red.	Prontosil red, pyridium, pyramidone, sulphonal.
Red in alkaline urine.	Phenolphthalein and phenol red.
Brownish-black.	Phenols, phenylhydrazine.
Greenish.	Santonin, guaiacum, methylene blue.
Blue.	Methylene blue.

Turbidity

Freshly voided urine is normally clear and transparent unless the specimen is an alkaline one passed after a heavy meal, when the turbidity is due to precipitation of earthy phosphates. Strict vegetarians may normally pass a turbid alkaline urine. Clear urine may become cloudy on standing due to precipitation of traces of mucoprotein (or nucleoprotein ?) derived from epithelial cells of the urinary tract. The conversion of urea to ammonia by bacteria may render a standing urine alkaline and turbid due to precipitation of phosphates. In concentrated acid urines, acid urates may cause turbidity which is removed on warming, Pathologically a urine might be opalescent, due to bacteria, or milky, due to fat (chyluria) or pus (pyuria).

Odour

The nature of the substances responsible for the usual odour of urine is unknown. Whatever they are, they are only present in minute amounts. The odour may be modified by ingestion of certain foods or drugs. This is most noticeable after eating asparagus ; the odour is then mainly due to methyl mercaptan. The ammoniacal smell of stale urine is due to the action of bacteria on urea. In starvation or incomplete fat oxidation, acetone bodies may modify the smell.

Reaction

The mixed samples of normal urine over twenty-four hours are ordinarily acid, approximately pH 6. Individual samples may vary considerably from pH 4·8 to 7·4. Although there are a number of acids and bases in urine, the reaction is probably mainly dependent upon the relative proportions of the acid and alkaline phosphates of sodium and potassium. It will be recalled (pp. 15, 298), that in plasma alkaline phosphate predominates, but the kidneys are able to retain part of the base for the alkali reserve and excrete a predominantly acid phosphate. Fixed base can also be conserved by the production of ammonia in the kidneys from urea.

The most important factor in determining the pH of urine is the food. In general, foods which give an acid ash (meats, eggs, cereals) cause excretion of acid urine, whereas milk and most fruits and vegetables which give an alkaline ash decrease the elimination of acid, and may even give an alkaline urine. Herbivorous animals and strict vegetarians may normally pass an alkaline urine; carnivorous animals and men on a mixed diet pass an acid urine.

The reaction of urine, however, varies at different times of the day. The observation that a neutral or alkaline urine might be passed an hour or so after a meal, and especially a high protein meal, gave rise to the suggestion that this so-called *alkaline tide* was a compensation for the acid secreted in the gastric juice (the compensating secretion of base in pancreatic juice about the same time was not considered). Since acid urines were found to be secreted during sleep, and alkaline urines frequently observed after rising, the activity of the respiratory centre in removing CO_2 was correlated with the reaction of the urine. Recent investigation has revealed the existence of so many factors which may affect the pH of urine that it is very doubtful whether there is any direct relation between the reaction of urine and the secretion of gastric juice, or the *normal* activity of the respiratory centre.

The acidity of urine may be increased after strenuous muscular exercise (elimination of lactic acid), by ingestion of ammonium salts of strong acids, *e.g.*, NH_4Cl, or by pathological conditions promoting acidosis. An alkaline urine may be produced by ingestion of sufficient $NaHCO_3$, or Na or K salts of organic acids, *e.g.*, citrates or tartrates, which are oxidised in the body to bicarbonate. Ammonium carbonate would not produce an alkaline urine, because ammonia is rapidly converted into urea.

Acid Excreted in Urine

An adult on a mixed diet normally excretes the equivalent of 500–900 c.c. N/10 acid per day. Larger amounts would be excreted on a high protein diet or in conditions producing acidosis. In severe diabetic acidosis 4,000–6,000 c.c. N/10 acid may be eliminated, indicating that the body is well adapted to cope with abnormal acidity. Acid is probably the most poisonous waste product of metabolism and is the cause of the coma of the final stages of diabetes mellitus and nephritis.

Only part of the excreted acid is in a form which can be titrated with alkali. The **titratable acidity** of urine is most logically * determined by finding the volume of N/10 NaOH required to bring the urine to pH 7·4, the normal pH of blood; phenol red is used as indicator. In this way the titratable acidity of urine from a mixed diet is usually between 200 and 400 c.c. N/10 acid per day. The remaining acid is excreted in the form of ammonium salts. Since an alkaline urine is practically free from ammonia, the urinary ammonia can be taken as a measure of the amount of acid which has been neutralised by ammonia formed for that purpose from urea by the kidneys. The ammonia + titratable acidity of a urine therefore gives a measure of the amount of excess acid which has been eliminated. The ammonia excretion on a mixed diet is between 300 and 600 c.c. N/10 per day. In the absence of renal disease, the ammonia content of urine is parallel to the acidity, a high acidity being associated with a high ammonia value and a low acidity with a small output of ammonia. Very high ammonia values have been recorded in diabetic acidosis (*e.g.*, over 3,000 c.c. N/10 per day).

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* Another common method, in which the urine is titrated to pH 8·5, using phenolphthalein as indicator, gives values about 20%–25% higher.

The amount of **organic acid** * excreted (partly as salts) is about 6 c.c. N/10 per kg. body weight in twenty-four hours. The greater part of this is excreted during the daytime and is probably derived from muscular activity and ingestion of food. The amount is greatly increased in acidosis (ketosis) from incomplete fat metabolism, due to the excretion of large quantities of acetoacetic and β-hydroxybutyric acids (*e.g.* in starvation or diabetes mellitus).

Quantitative Analysis of Urine

The *quantitative* analysis of a random sample of urine is a sheer waste of time and labour. The composition of specimens of urine passed at different times of the day is so variable that a dependable quantitative analysis can only be made on the mixture of all urine passed during twenty-four hours, usually from 6 a.m. to 6 a.m. the following morning. Only in this way can an idea of the daily excretion of a particular substance be obtained.

Owing to the action of organisms, certain urinary constituents may be partially decomposed when the voided urine is allowed to stand, even for two or three hours. Ammonium carbonate is very readily formed from urea in this way, so that after a urine has stood twenty-four hours, analytical values for urea, ammonia, pH and titratable acidity would be very misleading unless bacterial action has been prevented by the addition of a suitable preservative.

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* Estimated by titration after removal of phosphates and carbonates, a correction being made for bases, like creatinine.

The choice of a preservative depends on the substances to be estimated, since there is no known preservative suited to all analytical methods. Acidification (1 c.c. concentrated HCl per 100 c.c. urine) is excellent, provided the addition of acid does not interfere with the analysis. Toluene (sufficient to form an intact layer over the surface of the urine, which is well shaken with the toluene when the addition is made) is most commonly used for general purposes. Its chief disadvantages are that its " greasiness " may make accurate measurements in pipettes, etc., difficult and that it does not always check bacterial action. Other preservatives, *e.g.*, chloroform, thymol, formalin and mercuric chloride, are limited in their use on chemical grounds. Whatever preservative is chosen, it is better to keep the urine in a cool place, and, if possible, in a refrigerator. The difficulty of collecting and preserving twenty-four-hour samples of urine for quantitative analysis and the limited value of the results have led to a clinical preference for blood analyses for routine work. Quantitative urine analysis is of value, however, in special investigations and renal efficiency tests.

Composition of Urine

The composition of urine is extremely variable, even in a twenty-four-hour sample. The only constituents in which any degree of constancy may be expected are those of purely endogenous origin, *e.g.*, creatinine. In the table on p. 448 typical values for the urine of a healthy adult on a mixed diet are given. It must, however, be realised that values outside these would not necessarily be in any way abnormal and might only be due to a peculiarity in diet. An abridged table of values which should be memorised is given in the Appendix.

Variations in Composition due to Diet

The effect of diet on the composition of urine is well shown in the table on p. 449, which has been based upon figures given by

COMPOSITION OF NORMAL URINE

(*Typical values for twenty-four hours' output on a mixed diet*)

Constituent	Output in 24 Hours	Remarks	Approximate per cent.
Acetone bodies (total) .	20–50 mg.	—	0·002
Ammonia . . .	0·5–1·0 g.	300–600 c.c. N/10	0·05
Calcium (Ca) . .	0·1–0·3 g.	—	0·01
Chloride (as NaCl) .	10–15 g.	6–9 g. Cl.	0·9
Creatine . . .	*Nil* or traces.	Children, 10–15 mg.	0·0
Creatinine . . .	1·0–1·5 g.	—	0·1
Diastatic index . .	6·7–33·3	—	—
Glucose . . .	About 0·5 g.	—	0·03
Glucuronates (as acid) .	,, 150 mg.	—	0·009
Hippuric acid . .	,, 0·7 g.	—	0·05
Indican . . .	5–20 mg.	—	0·001
Iron (Fe) . . .	1–10 mg.	—	0·0005
Magnesium (Mg) . .	0·05–0·2 g.	—	0·01
Nitrate . . .	About 0·5 g.	—	0·03
Nitrogen (N)			
Total N . . .	About 16 g.	—	1·0
Urea N . . .	,, 14 g.	—	0·9
Ammonia N . .	,, 0·5 g.	—	0·03
Amino-acid N . .	,, 0·6 g.	—	0·04
Creatinine N . .	,, 0·6 g.	—	0·04
Uric acid N . .	,, 0·2 g.	—	0·015
Organic acids . .	About 6 c.c. N/10 per kg. body weight.	—	—
Oxalic acid . .	15–20 mg.	—	0·0015
pH	4·8–7·4	Average pH 6.	—
Phenols . . .	About 0·2 g.	—	0·015
Phosphate (P_2O_5) .	1–5 g.	2–6% organic.	0·17
Potassium (K) .	2–4 g.	—	0·17
Purine bases . .	16–20 mg.	—	0·0015
Sodium (Na) . .	3–6 g.	—	0·33
Solids (total) . .	55–72 g.	—	4·5
Specific gravity . .	1,010–1,025	—	—
Sulphur (SO_3)			
Total SO_3 . .	1·6–3·6 g.	To get S, multiply by 0·4.	0·17
Inorganic sulphate SO_3	1·4–3·3 g.		0·16
Ethereal sulphate SO_3	0·1–0·25 g.		0·014
Neutral sulphur as SO_3	0·15–0·4 g.		0·02
Titratable acidity . .	200–400 c.c. N/10.	—	—
Urea	20–35 g.	—	2·0
Uric acid . . .	0·1–2·0 g.	Average 0·7 g.	0·05
Volume . . .	1,000–1,800 c.c.	—	—

Compiled mainly from Harrison (Ref. 3) and Hawk and Bergeim (Ref. 5)

Bodansky (Ref. 1). The original figures have been amended so as not to distract attention by insignificant variations.

OUTPUT IN URINE IN GRAMS IN TWENTY-FOUR HOURS

	Adequate Dietary			Starvation	
	Ordinary Protein Intake	High Protein Intake	Low Protein Intake	First Day	Fourth Day
Nitrogen					
Total N . . .	13·0	23·0	4·2	7·0	14·0
Urea N. . . .	11·0	20·0	2·9	5·8	11·8
Ammonia N . . .	0·4	0·8	0·2	0·2	1·3
Creatinine N . . .	0·6	0·6	0·6	0·6	0·6 *
Uric acid N . . .	0·2	0·3	0·1	0·15	0·1
Sulphur					
Total SO$_3$ . . .	2·6	3·5	0·8	1·2	2·0
Inorganic SO$_3$ . .	2·1	2·8	0·6	—	—
Chlorides (NaCl) . .	12·0	15·0	10·0	5·2	1·3
Phosphates (P$_2$O$_5$) . .	2·6	4·0	1·0	1·7	1·1
Titratable acidity (c.c. N/10)	280	650	160	180	720
Acetone bodies . . .	—	—	—	—	3·9

* Includes 0·15 g. creatine N.

In a properly fed individual, urea is mainly a product of exogenous metabolism; in starvation, during the first day there is sufficient glycogen reserve to prevent the considerable breakdown of protein evidenced by the high nitrogen output on the fourth day. After the glycogen reserve is exhausted, the daily excretion of nitrogen falls progressively with the duration of starvation. (The professional faster, Succi, excreted about 3 g. after twenty days.) During starvation the fat reserves of the body are used as far as possible, but there is appreciable use of tissue protein. When the fat is exhausted, tissue proteins remain as the sole source of energy for the body. Since protein has only four-ninths of the energy value of fat, there will be a very extensive metabolism of protein, and consequently a big rise in nitrogen excretion, which, since it is a warning of imminent death, is often called the **premortal rise.**

The ammonia values reflect the acidity. The acid-forming nature of protein is clear. In starvation the high acidity on the fourth day is mainly due to ketosis, since a large amount of fat is being metabolised ; there is no ketosis on the first day, because the glycogen reserves have not been exhausted.

Creatinine is obviously a product of endogenous metabolism only. On the fourth day of starvation some creatinine is excreted as creatine.

Uric acid is partly endogenous in origin. On purine-rich diets uric acid nitrogen may rise to over 0·6 g.

The total sulphur, which is chiefly sulphate, is mainly exogenous in origin and its variations closely follow those of urea.

The retention of chloride during starvation is clearly shown on the fourth day.

THE INDIVIDUAL CONSTITUENTS OF URINE

To save repetition, information which has been recorded elsewhere is only given here in note form in the first paragraph after each heading. The figures are typical values for the amounts excreted in twenty-four hours.

Nitrogenous Constituents

Urea (30 g.). Chief end product of protein metabolism : mainly exogenous (pp. 264–269).

Uric Acid (0·7 g.). End product of purine metabolism : partly exogenous (pp. 290–292).

Uric acid occurs in urine in the form of urates, *e.g.*, monosodium urate. On acidifying urine, uric acid, which is only very sparingly soluble, separates in a variety of characteristic forms (see Fig. 39). The brick-red deposit formed on cooling concentrated urines is mainly acid urate. Pure uric acid is colourless ; deposits of uric acid and urates from urine are almost invariably coloured by adsorbed urinary pigments, particularly the red uroerythrin.

Creatinine (1·2 g.). End product of endogenous creatine metabolism in muscle ; creatine excreted by children and pregnant women (pp. 285–288).

The "*creatinine coefficient*," the creatinine nitrogen in mg. excreted per kg. per day, usually lies between 7 and 11.

Ammonia (0·7 g.). Defence against acid ; formed from urea in kidneys ; high in conditions associated with acidosis ; not excreted after ingestion of ammonium salts except those with strong acids ; liable to be formed from urea when urine is allowed to stand (pp. 153, 273, 444–446).

Hippuric Acid (0·7 g.). Benzoic acid detoxicated by conjugation with glycine (p. 315).

Fruits and vegetables contain benzoic acid or aromatic substances which form it on oxidation. Small amounts of benzoic acid might be formed from phenylalanine or tyrosine in the large intestine. The excretion is greater in herbivorous animals. Plums and prunes have a relatively high benzoic acid content, the latter about 0·1%.

Amino-acids (N = 0·6 g.). The amino-acids are present in such small amounts that only a few have been isolated, *e.g.*, cystine, tyrosine, leucine. Large amounts are only excreted when there is very extensive destruction of the liver, *e.g.*, acute yellow atrophy. Cystine in abnormal amount is excreted in the rare condition of cystinuria (p. 277).

Purine Bases (40 mg.). Small amounts of purine bases are derived, like uric acid, partly from exogenous and partly from endogenous (tissue nucleoprotein) metabolism. Their excretion is parallel to that of uric acid. The purines found in human urine, apart from adenine, guanine, hypoxanthine and xanthine, are 7-methylguanine (*epiguanine*), 1-methylxanthine, 7-methylxanthine (*heteroxanthine*) and 1, 7-dimethylxanthine (*paraxanthine*). The methyl xanthines are probably exogenous products derived from ingested caffeine or theobromine.

Allantoin (10 mg.). Only small amounts in man. End product of purine metabolism in most mammals (p. 291).

Indole-acetic Acid. A product of bacterial decomposition of tryptophan (p. 201).

Indole-acetic acid is probably responsible for the reddish appearance of urine which has been treated with mineral acid. The red pigment formed, **urorosein,** is supposed to be nitrosoindole-acetic acid. The reaction works best in stale urines which contain nitrite ; fresh urine may not give the reaction unless nitrite is added.

Sulphur Compounds

Sulphur is excreted in three forms :—

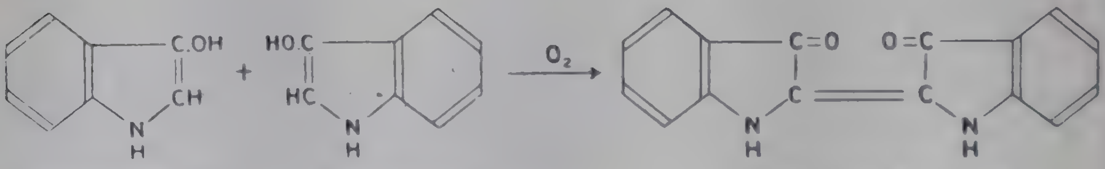
Inorganic sulphate } Total sulphate.
Ethereal sulphate }
Neutral sulphur.

The total sulphate represents chiefly sulphur of proteins which has been completely oxidised. Very little preformed sulphate is absorbed from ingested food. Total sulphate is, like urea, mainly derived from exogenous protein metabolism.

Inorganic Sulphate (2 g. SO_3) is in the form of Na, K, Ca, Mg and NH_4 sulphates and gives a precipitate of $BaSO_4$ directly from the urine when acidified $BaCl_2$ is added.

Ethereal Sulphate (0·2 g. SO_3) consists of the sulphuric esters of certain phenols (p. 316). They form no precipitate on addition of acidified $BaCl_2$. Ethereal sulphate gives an indication of the extent of phenol detoxication. Some of the phenols, but not necessarily all, are derived from putrefaction of protein in the large intestine (p. 201). In phenol poisoning all sulphate may be conjugated, and the urine in such a case may be free from inorganic sulphate.

Clinically, the ethereal sulphate of most interest is that of indoxyl, indican (" urinary indican "), which can be considered as arising from bacterial decomposition of tryptophan in the large intestine, or pathologically in decomposing pus anywhere in the body. Normally 5–20 mg. are excreted, the amount increasing in constipation. In disease (*e.g.*, cholera, typhus, gangrene of lung) sufficient indican may be excreted to cause the urine to assume a bluish tinge on the surface on standing. Indoxyl liberated from indican is oxidised to indigo blue on exposure to air.



INDOXYL INDOXYL INDIGO BLUE

The conditions which favour this reaction in urine are not known. Only a part of the indican forms indigo, presumably because the ethereal sulphate is not readily hydrolysed. This reaction is the basis of tests for indican. (Indoxyl is liberated from indican by strong HCl and treated with a suitable oxidising agent.)

Neutral Sulphur (0·3 g. SO_3). This heading is used for the grouping of a number of sulphur compounds found in urine.

Little is known of their origin, except that they are mainly products of endogenous metabolism. They are estimated in a group by finding how much additional sulphate is formed when the urine is treated with a powerful oxidising agent. They are sometimes described as *unoxidised sulphur*. Amongst the unoxidised sulphur compounds which have been identified in urine are cystine (p. 451), mercaptans (p. 203), thiocyanates (p. 318), derivatives of taurine, thiosulphates and urochrome. About 30 mg. of cystine are excreted per day.

Other Organic Compounds

Organic Acids. Apart from those already mentioned (*e.g.*, uric, hippuric, amino-acids), normal urine contains very small amounts of several organic acids, including volatile fatty acids (acetic, formic, butyric), lactic, oxalic, acetoacetic, β-hydroxybutyric, glucuronic (conjugated), glycerophosphoric, phenyl-acetic, phenyl-propionic, *p*-hydroxyphenyl-acetic, *p*-hydroxyphenylpropionic, and indole-acetic acids. The aromatic acids are intermediates in the bacterial decomposition of phenylalanine, tyrosine and tryptophan (p. 201).

Lactic acid excretion is increased by muscular exercise (p. 236).

Oxalic acid (20 mg.) is frequently found as calcium oxalate crystals (Fig. 35) in urinary deposits. The excretion is increased by ingestion of fruits and vegetables of relatively high oxalate content (*e.g.*, rhubarb, spinach). The oxalic acid normally present is probably partly unchanged ingested acid and partly derived from oxidation of fat or protein.

Acetoacetic and β-hydroxybutyric acids, see Acetone Bodies, p. 457.

Glucuronic acid, in the form of conjugated compounds, is present in traces in normal urine. The conjugated acids are formed in the detoxication (p. 316) of phenols produced by bacterial decomposition in the large intestine (p. 201). The amount is normally less than 0·15 g. per day. The amount is increased either by excessive intestinal putrefaction or by drugs which are detoxicated by glucuronic acid, *e.g.*, acetanilide, antipyrine, borneol, camphor, chloral, chloroform, menthol, morphine, naphthol, phenol, thymol and some sulphonamides, *e.g.*, sulphapyridine.

Glycerophosphoric acid and other organic phosphates form not more than 6% of the total phosphoric acid.

Carbohydrates. Ordinarily only traces of glucose are excreted in urine (not sufficient to give Fehling's or Benedict's tests). About half of the " sugar " of normal urine is unfermentable and probably represents traces of ingested sugars which cannot be utilised in the body. **Lactose** may be excreted in appreciable amounts by pregnant and lactating women. **Pentoses** may be excreted after ingestion of pentose-rich fruits (cherries, grapes, plums, prunes).

Phenols (0·2 g.). Phenols are excreted chiefly in conjugated form as ethereal sulphates (*q.v.*) or glucuronates.

Pigments. See p. 442.

Enzymes. Traces of many enzymes are found in urine, including an amylase, probably pancreatic amylase, pepsin, trypsin, and a lipase. Most attention has been given to the amylase, since the amount is much increased in pancreatic disease. Clinically, the amylolytic activity is recorded in terms of the **diastatic index,** which is the number of diastatic units in 1 c.c. of urine (a diastatic unit is the amount of amylase (diastase) which will digest 1 c.c. of 0·1% soluble starch solution in half an hour at 37° C.). The diastatic index, normally between 6·7 and 33·3, may rise to 200 or even 2,000 in acute pancreatitis.

Hormones and Vitamins. Certain hormones (*e.g.*, sex hormones) and vitamins (*e.g.*, B_1 and C) are found in urine. Attempts have been made to assess the vitamin needs of an individual by studying his urinary output after test doses. If he is well supplied with vitamin, a test dose should be largely excreted ; if his intake has been subnormal, he should retain most of the test dose. The method has met with some success in detecting mild deficiency of vitamin C.

Inorganic Constituents

Chlorides (12 g. NaCl). The large amount of chloride normally excreted is mainly due to chlorides ingested in food. Very low values are observed in starvation. In pathological conditions in which much fluid (which must be isotonic with blood) collects in the body, *e.g.*, pleural effusion, ascites, the œdemas of nephritis and cardiac disease, chloride excretion is very small.

Phosphates (2·5 g. P_2O_5). Relative proportions of BH_2PO_4 and B_2HPO_4 largely determine urine pH (p. 444).

The greater part of the phosphate excreted arises from ingested food which contains organic phosphates, *e.g.*, nucleoprotein, phosphoprotein, and phospholipides, as well as inorganic phosphates. Phosphates in food are not completely absorbed (p. 300), about one-third usually being excreted in the fæces. The smaller part of the urinary phosphate is derived from endogenous metabolism of phosphorus compounds. Pathologically, an increased excretion is observed in certain bone diseases, and, it is stated, in wasting diseases of the nervous system.

In neutral or alkaline urines, phosphates of Ca or Mg are often deposited (see p. 457).

Sulphates. See p. 452.

Bicarbonates. Small amounts of bicarbonate are usually present in urine. Appreciable amounts are only present in alkaline (usually turbid) urines. (Ammonium carbonate is rapidly formed when urine is allowed to stand, owing to bacterial decomposition of urea.)

Nitrates. The small amounts of nitrate represent nitrates ingested with food and excreted unchanged. Nitrates are not formed in the body. When urine stands, nitrite is formed from nitrate by bacterial action.

Sodium (5 g.) **and Potassium** (2 g.). The amount of Na and K excreted depends mainly on the diet. The proportion of potassium is increased on a large meat diet. In starvation more potassium is excreted than sodium, due to breakdown of muscle tissue which is relatively rich in potassium.

Calcium (0·2 g.) **and Magnesium** (0·15 g.). These elements in food are not completely absorbed (p. 299). Their urinary excretion is no indication of the amount absorbed, since they can also be excreted into the large intestine. Probably not more than 40% of total output of Ca is excreted in urine. The excretion of Mg is similar.

Ammonium. See p. 450.

Iron (5 mg.). Only traces of iron are excreted in urine. Part is in organic combination. For the excretion of iron see p. 310.

ABNORMAL CONSTITUENTS OF URINE

Protein. The traces of protein present in normal urine are not detectable by the ordinary simple tests. Pathologically, several proteins have been found in urine, *e.g.*, serum albumin, serum globulin, hæmoglobin, mucus, proteose, Bence-Jones protein.

The first four respond to the usual clinical tests for protein. The difficulty of differentiating them, for they do not always appear singly, has led to the somewhat indiscriminate use of the term "albuminuria."

The commonest form of albuminuria is undoubtedly due to escape of plasma proteins, *mainly albumin*, through the kidneys, *e.g.*, in nephritis, orthostatic albuminuria. There are so many possible causes of proteinuria of essentially pathological origin that a discussion is beyond our scope.

Globulin is usually found along with albumin. The albumin : globulin ratio varies greatly in different conditions, although it is usually higher than 2 : 1 (the ratio in plasma, see p. 158). The occurrence of globulin alone has been reported.

Hæmoglobin is usually present as a result of *hæmaturia* (red corpuscles in urine), due to hæmorrhage from the kidneys or urinary tract. If much blood is passed there may be sufficient fibrinogen to cause clotting. Hæmoglobinuria without hæmaturia is less common and is due to conditions of excessive hæmolysis.

Mucus. This is the most convenient term for an unidentified protein precipitated by acetic acid in the cold. It is probably a mucin rather than a nucleoprotein. The traces found in normal urine are increased in infections of the bladder.

Proteose may be found occasionally, but is of little clinical significance.

Bence-Jones protein is a rare form which is excreted in multiple myelomatosis of bone marrow and is of great value in diagnosis of the condition. This protein has the distinctive property of giving a precipitate when heated to a relatively low temperature (40°–60° C.), redissolving on further heating (100° C.), and separating again on cooling.

Peptonuria probably does not occur. The "biuret" reaction upon which the presence of peptone has been based is attributed by Godfried to a reaction of one of the urinary pigments.

Glucose. Easily detectable glycosuria may be due to several causes (see p. 228). In diabetes mellitus, urine may contain 3%–5% of glucose ; in severe cases, 100 g. of glucose may be eliminated in twenty-four hours. Fructose is sometimes excreted along with glucose in this condition.

Lactose. See p. 454.

Pentose. Apart from alimentary pentosuria (p. 454), pentosuria is caused by a rare inborn error of metabolism, found particularly in Jews.

Glucuronic Acid. See p. 453.

Acetone Bodies. The causes of ketonuria are discussed on pp. 254, 255. Small amounts of acetone bodies are excreted normally, *e.g.*, in twenty-four hours, acetoacetic acid 9 mg., β-hydroxybutyric acid 25 mg. and acetone 3 mg. Pathologically, the amounts are greatly increased, especially that of β-hydroxybutyric acid, of which 75 g. per day may be excreted in severe diabetes mellitus. This acid always predominates and usually accounts for over 70% of the total acetone bodies.

Bile. Bilirubin may be found in the urines of obstructive or hepatic jaundice. Bilirubinuria is usually accompanied by the excretion of bile salts. Bile salts may be excreted in urine without bile pigment in certain stages in liver disease. Traces of bilirubin without bile salts may be excreted in conditions of excessive hæmolysis.

Urobilinogen and Urobilin. In conditions of excessive hæmolysis, *e.g.*, hæmolytic jaundice or pernicious anæmia, part of the bile pigment formed by breakdown of the hæmoglobin is excreted in urine as urobilinogen (and possibly urobilin) (p. 176). Urobilin is formed from colourless urobilinogen when the urine is exposed to air, giving the urine an orange colour. Abnormal amounts of urobilin may be found in liver disease or temporarily in constipation.

Porphyrins. See p. 174.

Homogentisic Acid. See Alkaptonuria, p. 279.

URINARY DEPOSITS

In a microscopical examination of the sediment of urine, the cells and casts (organised deposits) are usually of greatest diagnostic value, but crystals and amorphous chemical deposits may sometimes be helpful. The deposit is most conveniently collected by centrifuging the urine. The nature of the crystals observed depends upon the reaction of the urine from which they are deposited. The commonest deposits are phosphates, oxalates and urates (including uric acid), and are frequently seen in normal urines.

Phosphates are usually found in alkaline urines. The commonest is *ammonium magnesium phosphate*, NH_4MgPO_4, (*triple phosphate*), which appears in two characteristic crystalline forms. "*Knife-rest*" or "*coffin-lid*" crystals (Fig. 33) are deposited from slightly alkaline or even slightly acid urine; the *feathery* form (Fig. 34) separates from alkaline urine, especially urine in which ammonia has been formed by bacterial action. A less common form of phosphate is *calcium hydrogen phosphate* (*stellar phosphate*), which separates from slightly acid or alkaline urine in rosettes of long prisms. Amorphous calcium and magnesium phosphates may be deposited from alkaline urines. All these phosphates are soluble in acetic acid. The commonest cause of a deposition of phosphates (*phosphaturia**) is a change in reaction after the urine has been passed.

Calcium oxalate is most frequently found in acid urines, but may be found in alkaline urine. The crystals assume two forms, *octahedra* or "*envelopes*" (Fig. 35), or "dumb-bells." The octahedra, which are the commonest, are usually very small. Calcium oxalate is insoluble in acetic acid.

Urates are usually found in acid urines. Uric acid (Fig. 36) separates in a variety of forms, including prisms, whetstones, rosettes, barrels, hexagons and needles, which are nearly always pigmented ("*cayenne pepper deposit*"). The urates of Na, K, Ca and Mg are often amorphous and pigmented with uroerythrin ("*brick dust*"). Sodium and ammonium urates may appear as characteristic "*thornapple*" crystals (spheres with spines). Ammonium urate, which may be deposited from alkaline urine, also forms spheres. Urates are usually redissolved on warming the urine. The commonest cause of the deposition of urates (*lithuria*) is the cooling of urine after it has been passed.

Amongst other substances which may be occasionally seen in urinary deposits are :—

Cystine, colourless hexagonal plates; important in the diagnosis of cystinuria (p. 277).

Tyrosine, sheaves of fine needles; usually from cases of liver disease.

Leucine, yellow spherical masses; usually found with tyrosine.

Bilirubin, reddish-brown cubes and rhombic plates, or amorphous; from urines containing much bilirubin.

These are usually found in acid urines; alkaline urines may give :—

Calcium carbonate, spherical masses or dumb-bells; not common in human urines, but common in the alkaline urines of herbivorous animals.

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* This term has also been used to describe an excessive urinary excretion of phosphates.

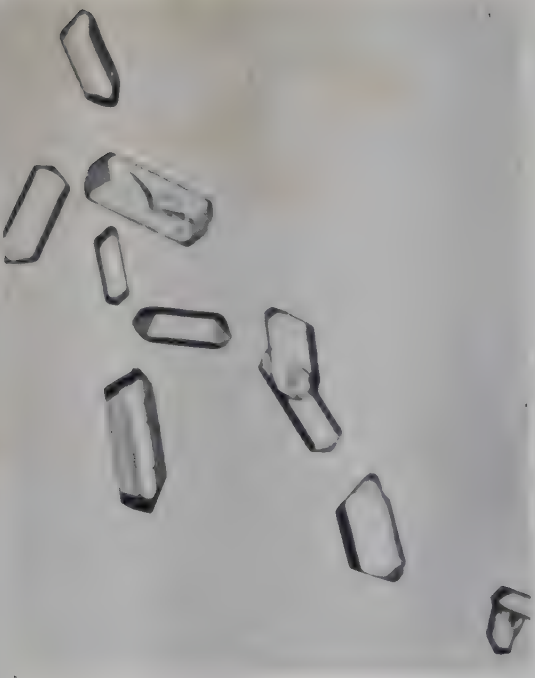

Fig. 33. Triple phosphate (knife-rest form). × 145.

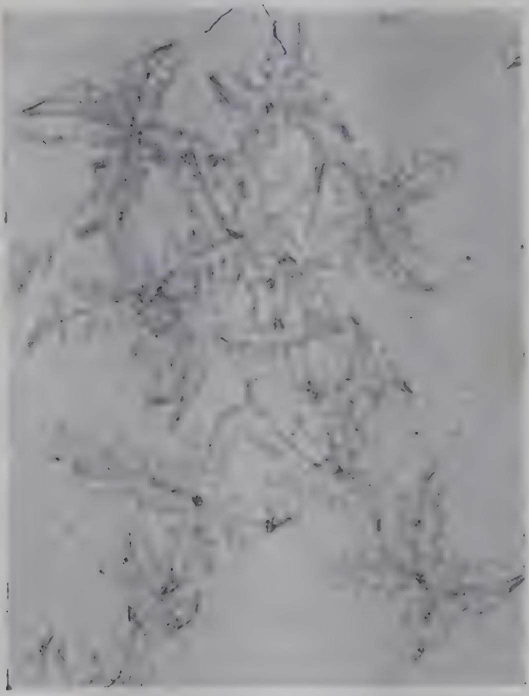

Fig. 34. Triple phosphate (feathery form). × 145.

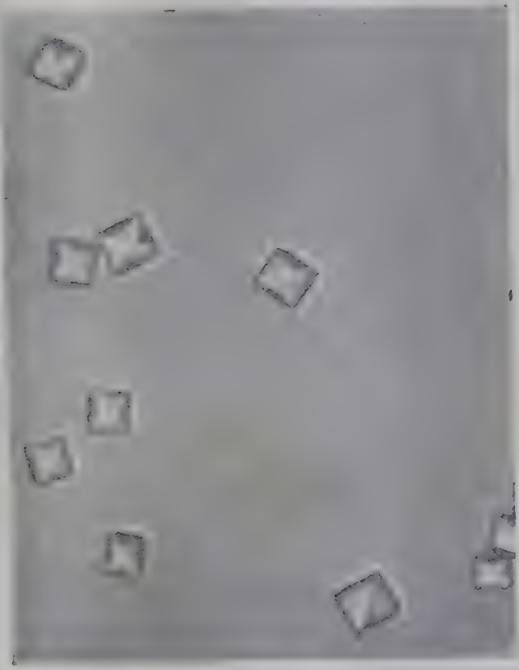

Fig. 35. Calcium oxalate (envelopes). × 640.

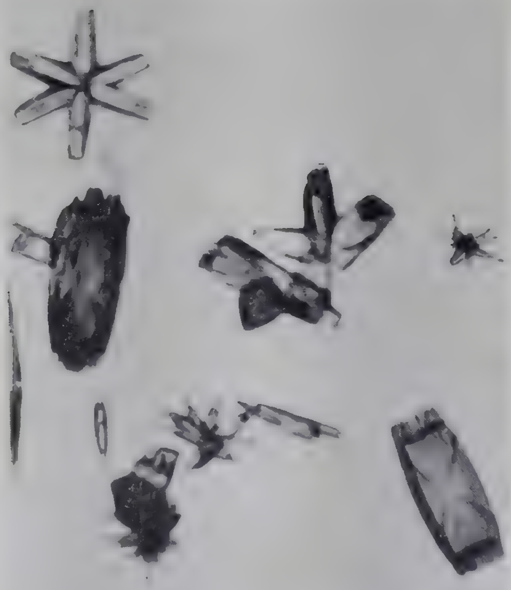

Fig. 36. Uric acid (barrels, stars, etc.). × 66.

Figs. 33-36. Urinary deposits.

Indigo, dark blue needles or amorphous ; from indican-rich urines after standing some time.

TESTS OF RENAL EFFICIENCY

Many methods have been used clinically for testing the efficiency of kidney function. In a given method only one particular function of the kidney is tested, but since in nearly all these tests conclusive results are only given when the kidney is seriously damaged, it is usually safe to infer general renal inefficiency. Most tests study the rate of excretion after large doses of normal metabolites, *e.g.*, urea or creatinine, or after doses of foreign substances, which are normally rapidly excreted by the kidneys unchanged, *e.g.*, dyes (phenol red, indigo carmine) or xylose. Several methods test the detoxicating power of the kidney, *e.g.*, the excretion of hippuric acid after a dose of benzoate (see p. 315). Other tests (*e.g.*, Mosenthal's) study excretion after a standard diet ; the urine is analysed for specific gravity, volume, total nitrogen and chlorides, day and night urine being separated. The tests most commonly employed are the urea and dye tests, the former in the *Urea Concentration Test*, in which the rate of excretion after a large dose of urea is measured, and in the *Blood Urea Clearance Test*, which measures the (theoretical) volume of blood which would be completely cleared of urea if clearance were complete. For details of the tests and their interpretation see Refs. 3, 5, 8.

2. FÆCES (3, 5)

Fæces are not merely undigested food residues. On certain diets, food residues may be scarcely detectable. The fæces passed in starvation have approximately the same composition as those passed under normal feeding. Food residues only become prominent in fæces when a large proportion of indigestible material, *e.g.*, cellulose, is eaten. The normal absence of easily digestible and absorbable food from fæces provides a valuable means of detecting impaired digestion and absorption in disease. Certain substances, *e.g.*, all metals except alkali metals, are excreted by the large intestine to a greater extent than by the kidneys.

Amount. The quantity of fæces varies from day to day and with the diet. Vegetable food, owing to the high proportion of indigestible cellulose, which also stimulates peristalsis, increases the bulk of fæces, whereas a meat diet which is largely absorbed diminishes the bulk. On a mixed diet an adult may pass from 60 to 250 g. of moist fæces containing 25–45 g. of solids in twenty-four hours, 100 g. of fæces usually being taken as the average figure. On a vegetable diet 370 g. may be passed, and while fasting 10–20 g. In young children the bulk is relatively large, *e.g.*, 60–150 g.

Composition of Fæces. Normal adult fæces which are fairly firmly moulded have a water content of 65–80%. Of the dry matter of fæces, approximately one-third is represented by bacteria. The rest consists of the remains of intestinal secretions, substances excreted by the large intestine (*e.g.*, Ca, Fe), cellular *débris* from the alimentary canal and small amounts of food residues. (For the changes which intestinal contents undergo after passing the ileocæcal valve see p. 199.) In health the food residues are predominantly indigestible residues, *e.g.*, cellulose (or food coated with cellulose, *e.g.*, uncooked starch grains), fruit skins, seeds, as opposed to digestible residues which have escaped absorption. In disease, fæces may contain appreciable quantities of absorbable food residues.

The only substances commonly estimated quantitatively in fæces are fat, nitrogen, and, in metabolic experiments, mineral elements. The water content of fæces is so variable that values for the constituents should be recorded, either as weight excreted per day, or as percentage of dry fæces. Dry fæces usually contain the following :—

Nitrogen	5–10%
" Fat "	10–20%
Ash	10–20%

Colour of Fæces. The normal brown colour of fæces is due to stercobilin (urobilin) formed by reduction of bile pigment (p. 176). The shade of brown is determined by the diet. Milk, cereals and chlorophyll-free vegetables give lighter, and meat, coffee, cocoa, blackberries, etc., darker stools. Excessive consumption of green vegetables may give a greenish tinge. Certain dyes which are not absorbed will colour fæces. Carmine is used in this way as a " marker " to indicate the beginning or end of a test period in metabolism experiments. Charcoal is used for the same purpose.

The fæces of newly born infants, **meconium,** is dark or blackish-green, due to biliverdin and porphyrin. The yellow stools of young infants on a milk diet contain bilirubin. As the bacterial flora develop in the intestine, more bilirubin is reduced to stercobilin, so that older children and adults normally pass no bilirubin in fæces.

In pathological conditions, the presence of blood may give the fæces either a tarry appearance or a red tinge, depending upon the position of the hæmorrhage into the gastro-intestinal tract. Excessive hæmolysis will cause abnormal excretion of stercobilin, and, therefore, very dark-brown fæces; a deficiency of bile pigments, as in obstructive jaundice, will give very pale fæces. Excess of fat will cause the fæces to be lighter in colour. The colour of fæces is also affected by certain drugs, *e.g.*, iron or bismuth tends to make them black, calomel green, rhubarb and senna yellow, methylene blue bluish.

Odour. The normal odour of fæces is mainly due to indole and skatole (p. 202). Mercaptans (*e.g.*, CH_3SH) and H_2S may contribute to the odour. A meat diet produces a more intense odour than a vegetable one, and a milk diet least of all.

Reaction. The normal reaction of fæces is slightly alkaline, pH 7·0–7·5, but may be slightly acid on a diet with a large proportion of carbohydrate or fat. Variations in health from pH 4·6 to 8·8 have been recorded.

Fat. The so-called " fat " of fæces includes all substances extracted from acidified fæces by ether. These substances are conveniently divided into two groups as follows :—

" TOTAL FAT "

" *Split Fat* "	" *Unsplit Fat* "
Preformed fatty acids. Fatty acids liberated from soaps.	Neutral fats. Phospholipides. Sterols. Pigments and other substances.

In the " unsplit fat," phospholipides and pigments, etc., form a very small proportion of the whole. In general, in a healthy adult on a mixed diet not more than 25% of the dried fæces should be " fat " ; of this " fat " more than 75% is normally " split." Sterols (mainly coprosterol formed by bacterial reduction of cholesterol, p. 86) account for 10–30% of the " unsplit fat."

The neutral fat is not undigested food fat. The amount is independent of the diet and may be unchanged even in starvation. Part of it is derived from the bacteria, and the rest is from breakdown of epithelial cells or is excreted into the large intestine. The " split fat " diminishes in amount on low-fat diets, and so may represent, at least in part, unabsorbed fatty acids from food. On low-fat diets, the proportion of " unsplit fat " in the total will, of course, be greater than 25%.

In disease, fæcal fat is increased when digestion or absorption of fat is impaired, *e.g.*, when digestion occurs, but absorption is defective in obstructive jaundice (lack of bile) and in cœliac disease, or when digestion is impaired in pancreatic disease (lack of lipase); in the first examples there is a normal or increased proportion of " split fat," in the last example there is a high proportion of " unsplit fat."

Nitrogen. Fæcal nitrogen is normally very little affected by the amount of protein ingested, provided that the protein is well masticated and easily assimilated. (If meat is " bolted," or an excessive amount of indigestible vegetable protein is taken, the undigested residues will increase fæcal nitrogen.) An adult on a mixed diet, which does not include an undue proportion of indigestible protein, usually excretes about 1 g.* of fæcal nitrogen per day, about 0·75 g. on a nitrogen-free diet and 0·25 g. in starvation. This nitrogen is made up of unabsorbed nitrogenous substances, secreted (*e.g.*, mucus) or excreted (*e.g.*, bile pigment) into the alimentary canal, nitrogenous constituents of bacteria and small amounts of nitrogen compounds derived from unabsorbed food, *e.g.*, undigested fragments, indole, skatole.

In disease, fæcal nitrogen may be greatly increased by failure of digestion or absorption of protein, *e.g.*, in pancreatic disease (trypsin lacking).

Salts. Moist fæces contain about 2–3% of salts. Most abundant are calcium and phosphate; there are small amounts of magnesium, iron, sodium, potassium, chloride and sulphate. The amounts of calcium, magnesium and phosphate, in particular, depend upon the diet, since these are only partially absorbed

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* In metabolic experiments in which fæcal nitrogen is not determined, it is usual to assume that 1 g. or 1·3 g. of nitrogen is excreted daily in fæces, if microscopical examination fails to reveal abnormal amounts of undigested protein.

(pp. 299, 300), *e.g.*, the proportion of calcium is higher on a milk diet, and of magnesium on a meat diet.

Microscopical Examination. Clinically, some of the most valuable information can be obtained by microscopical examination of a suspension of fæces in saline (0·65% NaCl is usually used, since there are salts in fæces which would make a suspension with ordinary isotonic saline hypertonic). Amongst substances which can be identified in this way are meat fibres in varying stages of digestion, fat globules, crystals of fatty acids and soaps, starch grains, cellulose residues, mucus, red blood corpuscles and other cells. Crystals of ammonium magnesium phosphate, calcium oxalate and cholesterol may also be seen.

3. SWEAT

Sweat is more dilute than any other fluid secreted by animals, and is always hypotonic. Its composition is variable, not only under different conditions, but in different parts of the body. Sweat as ordinarily secreted is acid in reaction (about pH 4·5), but if the skin is carefully washed and dried, sweat subsequently secreted is slightly alkaline (pH 7·0–7·4). It has been suggested that the acid reaction of sweat is due to contact with the skin. (Apocrine sweat, secreted from the axilla or groin, is ordinarily alkaline.) Sweat contains most of the diffusible constituents of plasma. The most abundant constituent is sodium chloride, considerable quantities of which may be eliminated, *e.g.*, 2–3 g. per hour, although the concentration is always less than that of plasma. The lactic acid content exceeds that normally found in blood. The table gives ranges compiled from various sources.

Composition of Sweat (Mg. per 100 c.c.)

Total solids	260–780
Organic matter	30–290
Ash	140–560
Urea	23–46
Lactic acid	70–160
Sugar	4–40
Chloride (NaCl)	55–480
Potassium	14–39
Calcium	21–23

APPENDIX

1. IMPORTANT DATA WHICH SHOULD BE MEMORISED

The values given below are typical values which have been selected as being the easiest to remember. In most cases considerable variations from these values are observed in healthy subjects. These ranges are given in the text, and should be examined when the approximate values given below have been memorised.

Blood

pH	7·4
Calcium (plasma)	10 mg. per 100 c.c.
Inorganic phosphorus (plasma)	4 ,, ,, ,,
Sugar	100 ,, ,, ,,
(Renal Sugar Threshold)	180 ,, ,, ,,
Urea	30 ,, ,, ,,
Hæmoglobin	14 g. ,, ,,
Plasma proteins	7 g. ,, ,,
Albumin : Globulin	2 : 1
Blood volume	5½ litres.
Plasma volume	3 ,,

Urine (excretion in twenty-four hours)

Volume	1,500 c.c. or 50 oz. or 1 c.c. per minute.	Urea	30 g.
		Ammonia	0·7 g.
Total solids	60 g. or 2 oz.	Uric acid	0·7 g.
S.G.	1,020	Creatinine	1 g.
pH.	6	NaCl	15 g.
Total acid	700 c.c. N/10	Phosphate (P_2O_5)	3 g.
Total nitrogen	15 g.	Sulphate (SO_3)	2 g.

Fæces (excretion in twenty-four hours)

Amount	100 g. (moist).
Nitrogen	1 g.
"Fat"	5 g.

2. IMPORTANT DATA RELATING TO NUTRITION

	Calorific Value	Respiratory Quotient	Specific Dynamic Action
Carbohydrate	4·1	1·0	5–6%
Fat	9·3	0·71	4%
Protein	4·1	0·81	30%
Mixed diet	—	0·85	10–12%

3,000 Calories are required in the food of the "average man" per day.

The **Basal Metabolic Rate** of the "average man" is 40 Calories per square metre body surface per hour.

The **Basal Metabolic Rate** of the "average woman" is 37·5 Calories per square metre body surface per hour.

Approximate Composition of Milk (g. per 100 c.c.)

	Protein	Fat	Carbohydrate
Cow's milk	3	4	5 } Very variable
Human milk	1	4	7 } Very variable

3. TEMPERATURE

Conversion of °C. into °F.
Multiply by 9, divide by 5 and add 32.

Conversion of °F. into °C.
Subtract 32, multiply by 5 and divide by 9.

	°C.	°F.
Freezing-point of water	0·0	32·0
Temperature of maximum density of water	4·0	39·2
Comfortable room temperature	15·6	60·0
Body temperature	36·9	98·4
Boiling-point of water at 760 mm.	100·0	212·0

4. PREFIXES USED IN THE METRIC SYSTEM

Smaller		*Larger*	
Micro	= one-millionth.	Deca	= ten.
Milli	= one-thousandth.	Hecto	= one hundred.
Centi	= one-hundredth.	Kilo	= one thousand.
Deci	= one-tenth.	Mega	= one million.

E.g., 1 Microgram (μg or γ) = 10^{-6} grams.
1 Micrometre or micron (μ) = 10^{-6} metres = 10^{-3} millimetres.
1 Millimicrometre or millimicron ($m\mu$) = 10^{-9} metres.
= 10^{-6} millimetres.
1 Hectolitre = 100 litres.

5. CONVERSION TABLE FOR WEIGHTS AND MEASURES

British Weights are Avoirdupois unless Specified

1 Angström unit = 0·1 millimicrons.

1 Atmosphere = { 33·9 feet of water at 4° C.
1033·3 grams per sq. centimetre.
760 millimetres of Hg at 0° C.
14·7 pounds per sq. inch.

5. CONVERSION TABLE FOR WEIGHTS AND MEASURES—*continued*

Calories (large)	=	1,000 small calories.
		41·8 × 10^{10} ergs.
		426·7 kilogram metres.
1 Centimetre	=	0·0328 feet.
		0·394 inches.
1 Centimetre of Hg at 0° C.	=	0·446 feet of water at 4° C.
		5·352 inches of water at 4° C.
		0·1934 pounds per sq. inch.
1 Cubic centimetre	=	0·061 cubic inches.
		0·2816 fluid drachms.
		0·2705 U.S. fluid drachms.
		16·9 minims.
		16·23 U.S. minims.
		0·0352 fluid ounces.
		0·0338 U.S. fluid ounces.
1 Cubic foot	=	28·317 litres.
		1,728 cubic inches.
		6·23 gallons.
1 Cubic foot of water at 62° F.	=	62·335 pounds.
1 Cubic inch	=	16·387 cubic centimetres.
1 Cubic metre	=	35·315 cubic feet.
		999·973 litres.
1 Cubic yard	=	27 cubic feet.
		0·76455 cubic metres.
		764·54 litres.
1 Drachm (apoth.)	=	2·194 drams.
		60 grains.
		3·888 grams.
1 Drachm (fluid)	=	3·5515 cubic centimetres.
		0·961 U.S. fluid drachms.
		60 minims.
1 Drachm (U.S. fluid)	=	3·697 cubic centimetres.
		1·041 fluid drachms.
		60 U.S. minims.
1 Dram (avoir.)	=	0·4558 apoth. drachms.
		1·772 grams.
1 Foot	=	30·48 centimetres.
1 Foot of water at 4° C.	=	2·2418 centimetres of Hg at 0° C.
		0·8826 inches of Hg at 0° C.
		0·4335 pounds per sq. inch.
1 Gallon	=	0·1605 cubic feet.
		277·4 cubic inches.
		1·201 U.S. gallons.
		4·546 litres.
		160 fluid ounces.
		8 pints.

5. CONVERSION TABLE FOR WEIGHTS AND MEASURES—*continued*

1 Gallon of water at 62° F.	=	10 pounds.
1 U.S. Gallon	=	0·1337 cubic feet. 231 cubic inches. 0·8325 Imp. gallons. 3·785 litres. 128 U.S. fluid ounces. 8 U.S. pints.
1 U.S. Gallon of water at 62° F.	=	8·338 pounds.
1 Grain	=	64·8 milligrams.
1 Gram	=	0·2572 apoth. drachms. 0·5644 drams. 15·432 grains. 0·03215 apoth. ounces. 0·03527 ounces.
1 Inch	=	2·54 centimetres.
1 Kilogram	=	32·151 apoth. ounces. 35·274 ounces. 2·2046 pounds.
1 Kilogram per sq. centimetre	=	0·9678 atmospheres. 73·556 centimetres of Hg at 0° C. 32·809 feet of water at 4° C. 14·223 pounds per sq. inch.
1 Litre	=	1000·027 cubic centimetres. 0·0353 cubic feet. 61·025 cubic inches. 0·22 gallons. 0·2642 U.S. gallons. 35·196 fluid ounces. 33·814 U.S. fluid ounces. 1·76 pints. 2·113 U.S. pints.
1 Metre	=	3·281 feet. 39·37 inches. 1·094 yards.
1 Millilitre	=	1·000027 cubic centimetres.
1 Minim	=	0·0592 cubic centimetres. 0·961 U.S. minims.
1 U.S. Minim	=	0·0616 cubic centimetres. 1·041 minims.
1 Ounce (apoth.)	=	17·55 drams. 8 apoth. drachms. 480 grains. 31·103 grams. 1·097 ounces.

5. CONVERSION TABLE FOR WEIGHTS AND MEASURES—*continued*

1 Ounce (avoir.)	=	16 drams. 7·292 apoth. drachms. 437·5 grains. 28·35 grams. 0·9115 apoth. ounces.
1 Ounce (fluid)	=	28·412 cubic centimetres 1·734 cubic inches. 8 fluid drachms. 480 minims. 0·961 U.S. fluid ounces.
1 U.S. Ounce (fluid)	=	29·573 cubic centimetres. 1·805 cubic inches. 8 U.S. fluid drachms. 480 U.S. minims. 1·041 fluid ounces.
1 Pint	=	34·675 cubic inches. 0·56825 litres. 20 fluid ounces. 19·2 U.S. fluid ounces. 1·2 U.S. pints.
1 U.S. Pint	=	28·875 cubic inches. 0·4732 litres. 16·65 fluid ounces. 16 U.S. fluid ounces. 0·8327 pints.
*1 Pound (apoth.)	=	5,760 grains. 373·24 grams. 12 apoth. ounces. 13·166 ounces.
1 Pound (avoir.)	=	7,000 grains. 453·59 grams. 14·583 apoth. ounces. 16 ounces. 350 scruples.*
1 Pound per sq. inch	=	70·31 grams per sq. centimetre. 2·307 feet of water at 4° C.
1 Quart	=	1·1365 litres. 40 fluid ounces. 1·201 U.S. quarts.
1 U.S. Quart	=	0·9463 litres. 32 U.S. fluid ounces. 0·8327 quarts.
*1 Scruple	=	0·3333 apoth. drachms. 0·731 drams. 20 grains. 1·296 grams.

* Now practically obsolete.

5. CONVERSION TABLE FOR WEIGHTS AND MEASURES—*continued*

*1 Scruple (fluid) = 1·1838 cubic centimetres.
20 minims.

1 Square centimetre = 0·001076 sq. feet.
0·155 sq. inches.

1 Square foot = 929·03 sq. centimetres.
144 sq. inches.

1 Square inch = 6·4516 sq. centimetres.

1 Square metre = 10·764 sq. feet.
1,550 sq. inches.
1·196 sq. yards.

1 Square yard = 1,296 sq. inches.
0·836 sq. metres.

1 Stone = 6·35 kilograms.
14 pounds.

1 Yard = 91·44 centimetres.

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* Now practically obsolete.

6. APPROXIMATE ATOMIC WEIGHTS OF COMMON ELEMENTS

Aluminium	27	Copper	63·6	Nitrogen	14
Antimony	121·8	Fluorine	19	Oxygen	16
Arsenic	74·9	Gold	197·2	Phosphorus	31
Barium	137·4	Hydrogen	1	Platinum	195·2
Bismuth	209	Iodine	126·9	Potassium	39·1
Boron	10·8	Iron	55·8	Silicon	28·1
Bromine	79·9	Lead	207·2	Silver	107·9
Calcium	40·1	Lithium	6·9	Sodium	23
Carbon	12	Magnesium	24·3	Sulphur	32·1
Chlorine	35·5	Manganese	54·9	Tin	118·7
Chromium	52	Mercury	200·6	Zinc	65·4
Cobalt	58·9	Nickel	58·7		

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